January 2019

Neurodevelopmental Risks Of Non-Syndromic Craniosynostosis

Robin T. Wu

Neurodevelopmental Risks of Non-syndromic Craniosynostosis

A Thesis Submitted to the

Yale University School of Medicine

In Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

By

Robin T. Wu

2019

Neurodevelopmental Risks of Non-syndromic Craniosynostosis

Robin T. Wu, Kyle S. Gabrick, Andrew T. Timberlake, Anusha Singh, Paul F. Abraham, James Nie, Taylor Halligan, Raysa Cabrejo, Derek M. Steinbacher, Michael Alperovich, John A. Persing, Yale University, School of Medicine, New Haven, CT.

Purpose: Nonsyndromic craniosynostosis may manifest with complex cognitive, language, behavioral, and emotional sequelae, depending on the suture fusion involved. De-novo or rare transmitted mutations in the *SMAD6* gene affect midline synostosis in 7% of patients. Current standards of assessment, such as the Bayley Scales of Infant Development (BSID), may not predictive of long-term development, paving the way for newer assessments such as functional magnetic resonance imaging (fMRI) and the event related potentials (ERP), which measures passive neurological responses to speech sounds.

Methods: Cranially-mature, post-operative unilateral coronal, metopic, midline SMAD6 mutated and age/race/gender/synostosis/operation matched non-SMAD6 controls from the Yale Craniofacial Clinic and the Children's Hospital of Philadelphia (CHOP) completed a double-blinded neurodevelopmental assessment, which included the Wechsler Fundamentals, Wechsler Abbreviated Scale of Intelligence, and Beery-Buktenica Developmental Test. Unilateral coronal (ULC) or metopic synostosis were age/gender/handedness matched to controls and participated in a GoNoGo task under fMRI. Craniosynostosis infants were given the BSID and ERP testing at two points (pre and post operatively), and after they reached $\geq$6 years of age, patients completed the Wechsler Abbreviated Scale of Intelligence and Wechsler Fundamentals to measure 5 language functional domains.

Results: ULC patients had a mean verbal IQ of 117.3 and performance IQ of 106.4, performed above average on academic achievements except for numerical, but below average on all visual-motor tests. Right ULC had improved spelling compared to left ULC, controlled for exogenous influences ($p=0.033$). Metopic patients with mild phenotype (endocranial bifrontal angle <124) performed better in word reading ($p=0.035$) and reading composite ($p=0.014$) than patients with severe stenosis ($\geq$124). After controlling for exogenous factors, midline synostosis patients with SMAD6 mutations performed worse on numerical operations($p=0.046$), performance IQ($p=0.018$), full IQ($p=0.010$), and motor coordination($p=0.043$) than those without the mutation. Among seven ULC and six metopic patients that participated in fMRI, metopic patients had decreased blood-oxygenation-level-dependent signal in the posterior cingulate($p=0.017$) and middle

temporal gyrus(MTG;p=0.042). ULC had decreased signal in the posterior cingulate(p=0.023), MTG(p=0.027), and thalamus(p=0.033), but increased signal in the cuneus(p=0.009) and cerebellum(p=0.009). Among 10 craniosynostoses patients who received ERP/BSID testing in infancy followed by school-age neurocognitive testin, the left frontal ERP cluster strongly correlated with word reading (R 0.713, p=0.031), reading comprehension (R 0.745, p=0.021), and language composite scores (R=0.771, p=0.015). Correlations for BSID cognitive, expressive language, and language composite scores had no predictive value ($R<0.5$, $p>0.05$) for neurocognitive scores.

Conclusions: Post-operative cranially mature ULC patients have higher verbal IQ scores, but worse mathematical and visual-motor achievement. Left-sided ULC patients may perform worse in spelling. The severity of orbito-frontal dysmorphology in metopic synostosis significantly impacts long-term cognitive function and academic achievement. Neuropsychiatric development may be in whole or in part under genetic control. SMAD6 mutations led to poorer performance on mathematics, performance-IQ, full-IQ, and motor coordination, even after controlling for exogenous factors. ULC patients may have emotional dyregulation in response to frustration while metopic patients may have attenuated emotional reactions. ERP assessment in nonsyndromic craniosynostosis patients has significantly better predictive value for future neurocognitive assessment than the standard BSID test. Use of ERP assessment may help tailor treatment for language deficits earlier in development.

Acknowledgements

I would like to thank my co-authors for their tireless contributions to this work and my faculty mentors for their belief in me. I am so lucky to have gotten the chance to participate in research with Dr. John Persing on work for which he has pioneered and changed the field of craniosynostosis.

I am grateful for my wonderful friends and future colleagues in medical school Alyssa Zupon, Rebecca Fine, Elliot Morse, Matthew Swallow, Tejas Sathe, and Brandon Sumpio, for surviving all the long rotations and late nights with me.

A very special thank you to Pranavi Vemuri, Yohan Perera, Jessica Shepis, and Jeffrey Chen for their lifelong friendship.

Finally, I give all my love to my mom and dad, my life coaches and perpetual cheerleaders.

This work was completed with monetary contributions from the Plastic Surgery Foundation (Award Number: 513938).

Table of Contents

Introduction

Non-syndromic Craniosynostosis

Cranial growth is governed by complex interactions between the brain, dura mater, cartilaginous sutures, and bony plates.[1] Patent calvarial sutures permit the skull to accommodate rapid expansion of the underlying brain in early infancy. Physiologic closure follows a conserved sequence; the posterior fontanelle obliterates between 1-3 months, followed by the metopic suture between 3-8 months, the anterior fontanelle between 9-18 months, and the remainder of sutures in adulthood.[2]

Premature fusion of calvarial sutures restricts skull growth perpendicular to the affected suture[3]. This pathology, known as non-syndromic craniosynostosis, affects 1 in every 2000 to 2500 births[4]. Presentations are varied based on suture type but yield reliable phenotypes.

Ossification of midline calvarial sutures, metopic or sagittal nonsyndromic craniosynostosis, predicates abnormal skull growth in the anteroposterior direction and comprise the vast majority of cases.[5-7] Sagittal synostosis patients have stereotypical scaphocephaly, resulting in compensatory growth in the frontal/occipital regions and limited anteroposterior width.[8] Metopic synostosis is characterized by trigonocephaly, bitemporal narrowing, and orbital hypotelorism. The orbito-frontal dysmorphology includes symmetric supra-orbital retrusion with a keel-shaped deformity in the

midline.[9-12] Unilateral coronal craniosynostosis (ULC) is the next most common, with a prevalence of 66 per million children born.[13-15] Unilateral coronal synostosis (ULC) limits the frontal cranium asymmetrically and is characterized by ipsilateral forehead flattening, a shallow orbit, and a recessed supraorbital rim, often with contralateral frontoparietal bossing.[16,17] The rarest form of craniosynostosis is lambdoid fusion, comprising only 1-5% of craniosynostoses. Lambdoid synostosis results in ipsilateral occipital flattening and mastoid bossing.[18]

Surgical Correction of Craniosynostosis

Patients who undergo treatment prior to three months of age may be offered strip craniectomy by some centers with selective use of postoperative cranial orthoses.[1,19-21] At this vulnerable age, emphasis is placed on limiting blood loss and operative time.[20] Strip craniectomy relies on subsequent brain growth to yield skull expansion and improved cranial shape. After six months, the cranium begins to ossify and skull bones lose malleability. In these older patients, with some institutional exceptions, cranial vault remodeling is generally preferred for more predictable outcomes.[22]

Choice in surgical technique involves an array of variables including type of fused suture, clinical severity, patient age and comorbidities, and perspectives regarding neurologic development.[23,24] Controversy exists regarding the timing of surgical repair and indications for cranial vault remodeling versus strip craniectomy. Strip craniectomy is less invasive but cranial vault remodeling (CVR) carries the advantage of more

complete correction of the deformity and release of brain compression post-operatively, which may have a positive influence on brain development.[25,26]

Long-Term Neurodevelopmental Outcomes

Premature fusion of calvarial sutures, or nonsyndromic craniosynostosis has direct sequelae on abnormal skull growth and deformation of underlying brain structures.[5-7] While study results are varied, current literature has suggested that long-term neurodevelopmental sequelae may exist in up to 50% of nonsyndromic craniosynostosis patients.[7,26-29] Treatment goals for nonsyndromic craniosynostosis are two-fold: normocephaly of skull shape and improved long-term functional neurocognitive outcomes.[25,27,30] Surgical treatment can improve global cognitive development and IQ, however, recent scrutiny has revealed persistence of subtle learning deficits.[7,26-28]

Children born with craniosynostosis typically have normal global intelligence, but have speech and or language impairments. Magge et al. tested 16 children aged 6 to 16 years with surgically corrected sagittal synostosis, and found despite normal intelligence scores, 50% were diagnosed with at least one language related learning disorder.[7] Similarly, Shipster et al. tested 75 children aged 9 months to 15 years with sagittal synostosis and found no global cognitive impairment.[31] However, 37% had speech and/or language impairment, with expressive language being most frequently affected. Naran et al. reported a series of 101 patients, aged 2-18 years, in which a majority had

metopic pathology.[32] Abnormal language development was identified in 1 in 1.7 patients and speech therapy was necessary in 1 in 3.4 subjects. Chieffo et al. studied 65 children, 9 to 16 years of age, and found 30% of unicoronal synostoses patients to be comorbid with speech delays.[28]

Different sutures govern particular patterns of brain restriction. Thus, neurocognitive outcomes may vary based on suture fusion. The metopic and coronal sutures, in particular, are positioned in the anterior cranium. The adjacent frontal brain region is tasked with executive function, impulse inhibition, and personality.[33] Lesions are classically associated with emotional dysregulation such as depression, anxiety, aggression, and social inappropriateness.[34] Of particular interest, the limbic system leads emotional processing, comprising areas such as the cingulate cortex involved in stress processing.[35] A plausible hypothesis, then, would implicate metopic and coronal synostosis with frontal lobe associated behavioral deficits. Indeed, abnormally low frontal lobe volume and corpus callosum abnormalities in metopic patients has been hypothesized to predispose for cognitive, motor, verbal, attention, and visuospacial deficits.[26,36,37] Another study reported 30% of ULC patients demonstrated processing and planning speech delays.[28]

Shillito and Matson reported mental retardation rates of 2.6% in 66 ULC patients in 1968.[38] In 1977, Hunter and Rudd published up to a 10% retardation and 11% borderline personality rate in 52 patients with ULC.[38,39] Becker et al. documented 61% of right and 52% of left ULC had speech-language, cognitive, and/or behavioral aberrations, without

statistical difference, but did not review individual tests with more granularity.[40] Speltz et al. cognitively tested 28 ULC infants, mean age 6.5 months, pre-surgically and found no significant difference among sidedness or compared with other single-sutures synostosis.[41] Starr et al. tested synostosis infants between 17-19 months and similarly concluded that despite below-average performance among all subtypes, ULC patients were not distinguished by suture type or laterality.

Neurodevelopmental delays in patients with metopic synostosis range from 15% to as high as 61% and may be particularly severe.[11,42-45] The metopic suture, positioned exclusively in the anterior cranium, overlays adjacent frontal brain regions tasked with executive function, impulse inhibition, and personality.[33,34] Mendonca et al. found 30% of metopic synostoses patients had speech and language delays but denied correlation with craniometrics measurements.[46] Conversely, Bottero et al. reported 23% rate of developmental deficit in mild non-operative trigonocephaly and a 32% rate in more severe patients requiring surgical intervention.[36] With surgical correction, Kunz et al. claimed that among 40% of metopic children with delays pre-operatively, all either completely recovered or improved twelve months postoperatively.[47] One quantitative assessment of phenotypic severity measures the endocranial bifrontal angle.[12,48] Prior studies have identified increased cognitive deficits in infants with a more acute endocranial bifrontal angle using event-related potentials.[48]

However, neural plasticity and compensatory development complicate such conclusions and neurobehavioral variations may be subtle. Long-term influences on brain development and neurocognition require further investigation.[26,41,49-51]

Predictors of Neurodevelopmental Performance

Early detection and prevention is essential for cognitive remediation in nonsyndromic craniosynostosis patients. Therefore, there is a need for proper evaluative tools for predicting development. Younger age at surgical correction and more comprehensive surgical remodeling have been associated with better overall intelligence, reading skills, math, and visuomotor integration.[25,30]

Final volumetric cranial size and brain network fine-tuning are not reached until ages 7-11, suggesting neurocognitive testing should be performed at the time of cranial maturity.[52-54] Furthermore, neurocognitive testing is more sensitive for deficits at older ages given the increased neurocognitive demands of the cranially-mature cohort relative to toddlers.[55,56] While neurodevelopmental surveys have come a long way to categorize the rates of delay and the impact of surgery, these cognitively vulnerable patients may benefit from further risk stratification based on pre-operative phenotype.

Genetics in Craniosynostosis

Midline non-syndromic craniosynostoses are found to be under genetic influence. Common variants downstream of the BMP2 gene have been associated with sagittal

synostosis. Recent breakthroughs revealed that de novo or rare transmitted mutations in the SMAD6 gene, an inhibitor of BMP signaling, cause non-syndromic midline synostosis in 7% of patients.[57] Genetic interactions between SMAD6 mutations and the common BMP2 risk allele dramatically affect penetrance in these cases.

Bicoronal synostosis patients with FGFR3 mutations trended towards worse developmental and intellectual outcomes, though the differences did not achieve statistical significance.[58] Genomic analysis of intellectual disability by Lelieveld et al. identified the SMAD6 gene as a novel locus for intellectual disability, however the presence or absence of craniosynsotosis was not noted in children with SMAD6 mutations and intellectual disability.[59] Questions arise as to the effect of SMAD6 mutations on neurocognitive development in the setting of craniosynostosis, given that these mutations are the most frequent genetic cause of nonsyndromic craniosynostosis identified to date. While optimizing surgical interventions and pioneering new-age tests have proven efficacious in detecting neurocognitive risks in craniosynostosis, genetic risks are non-modifiable and easily tested.

Functional Magnetic Resonance Imaging in Craniosynostosis

Functional MRI (fMRI) has been efficacious in teasing out delicate brain dynamics. fMRI studies in craniosynostosis demonstrated altered connectivity in sagittal patients and resting state group differences among subtypes of synostosis.[60] Sagittal synostosis patients often demonstrate significant changes in the left supramarginal gyrus, which may

correspond to language related learning disorders.[61] Metopic patients exhibit more changes in the dorsolateral pre-frontal cortex which often impacts working memory and executive function. Unilateral coronal patients often have altered connectivity in the anterior prefrontal cortex which distort higher level thinking such as multi-tasking. Still, higher level emotional performances, such as stress and frustration, are more properly assessed with executive tasks.

Event Related Potentials in Craniosynostosis

In assessing development, the Bayley Scales of Infant and Toddler Development is the most popular and widely utilized measure of cognitive function in infants aged 1-42 months.[52,62] The output variable is a Mental Developmental Index (MDI), which comprises cognitive, language, motor, social-emotional, and adaptive behavior scales. Kapp-Simon et al. first began to assess mental development in craniosynostosis infants with and without treatment with the Bayley Scales of Infant Development.[49] They concluded that cranial reconstruction did not affect mental development, contradictory to much of the evidence now, which suggests that children often develop deficits in language and speech development, despite having intelligence scores in the normal range.[7] Recently, the predictive validity of this test has been called into question. Hack et al. pooled past MDI scores of 344 extremely low birth weight infants and compared them to the subjects' current school age cognitive functions; they found a poor positive predictive value of 0.37 for future IQ, calling into question the utility of this test.[53] It is

necessary, then, to develop a better predictor of future function, particularly with emphasis on language norms.

EEG studies are objective, non-invasive, non-sedative, and thus are considered the best way to study infant brain activity[63]. ERPs are convenient as they do not require overt behavioral/verbal response or even attention from the infant. Most ERP studies to date aim to elucidate neural networks of healthy infants with a growing field into pathologic identification of autism spectrum infants. Of the auditory ERPs, the P150/N250 components, two prominent deflections in the EEG waveform, have been extensively studied. Seery et al. identified atypical lateralization of these ERPs in infants at high risk for autism spectrum disorder[64]. Balan et al. also looked at these ERPs in plagiocephaly infants, finding attenuated P150/N250 amplitudes compared to controls[65].

The mismatch negativity (MMN) component of ERP is elicited by having an infant discriminate a deviant auditory stimulus in the context of repetitive 'normal' stimuli[66,67], and has been found to be clinically effective in predicting language acquisition. Infants are born with the ability to discriminate speech sounds from broad sources[68,69]. Between six and twelve months of age, in a process known as perceptual narrowing, infant's auditory perceptions specializes towards its native spoken language, virtually extinguishing non-native verbal phenome recognition[70,71]. Jansson-Verkasalo et al. suggested that delayed or atypical perceptual narrowing measured by retained MMN is longitudinally associated with delayed language skills at one and two years of age, which has since been verified by other electrophysiologic studies[71-73].

Our group was the first to look at ERPs in patients with craniosynostosis. Hashim et al. reported infants with nonsyndromic craniosynostosis have attenuated P150 waves in response to speech sounds compared with normal infants.[24] Yang et al. found that severe metopic synostosis, defined by an endocranial bifrontal angle less than 124^{o}, presented with attenuated P150 waves compared with controls while moderate metopic synostosis (greater than 124^{o}) had no difference. Recent work, not yet published, by Chuang et al. has reanalyzed results looking at the MMN waves pre and post-operatively. Preliminary results found that MMN waves are attenuated preoperatively in sagittal and severe metopic patients but then improve postoperatively. Thus, validation studies must be performed to assess the predictive value of ERP on nonsyndromic craniosynostosis patients.

Purpose

Treatment goals for non-syndromic craniosynostosis are based off of restoring aesthetic normocephaly and augmenting functional neurocognition. Unfortunately, due to the early age at intervention and difficulty assessing longitudinal outcomes, the field is plagued by knowledge gaps as to the long-term results in this patient population. As such, the purpose of this work was to outline the neurodevelopmental outcomes using traditional cognitive testing, new-age imaging, and craniometrics analysis. Results may be critical for predictive outcomes, patient counseling, and understanding the mechanism of disease.

Aim #1: To present the long-term neurodevelopmental profile of patients with unilateral coronal craniosynostosis. Among all subtypes of craniosynostosis, neurocognitive outcomes have not been well established for patients with unilateral coronal craniosynostosis. Additionally, this study seeks to identify the differential impact of right verses left sided fusion as well as the influence of exogenous factors in development.

Aim #2: To compare the long-term neurodevelopmental outcomes between patients with mild and severe metopic craniosynostosis. Earlier work from our lab has established an endocranial bifrontal angle of 124 degrees as the cutoff between mild and severe metopic craniosynostosis. We hypothesize that patients with more severe synostosis angles will have worse neurocognitive outcomes.

Aim #3: To compare the long-term neurodevelopmental outcomes between midline craniosynostosis patients with mutated *SMAD6* genotype and those with the wild type *SMAD6* allele. We hypothesize that patients with SMAD6 mutation will have residual decreased IQ and performance on academic achievement testing compared to unaffected individuals.

Aim #4: To characterize long-term emotional-response brain activity with the first-reported use of task-based fMRI analysis in unilateral coronal and metopic craniosynostosis. We hypothesize that coronal and metopic craniosynostoses will have different patterns of brain response to emotional stimuli compared to healthy matched controls.

Aim #5: To validate event related potential EEG testing in infancy with long-term language performance in craniosynostosis. Our lab began testing infants ten years earlier and we hypothesize that EEG testing in infancy can predict future language development.

Methods

Patient Selection and Individualized Testing Parameters

All testing was conducted with parental or legal guardian consent, patient assent/consent, and Institutional Human Investigations Committee approval. Patients treated at the Yale School of Medicine consistent with the inclusion and exclusion criteria specified for each study arm below were collected by the Yale Joint Data Analytics Team. Patients were excluded if they had any diagnosed neurological/developmental delay such as cerebral palsy or a Full-Score IQ [FSIQ] < 70. Patients with a documented or suspected syndromic craniosynostosis diagnosis were excluded.

Unilateral Coronal Craniosynostosis Categorization

Due to the low prevalence of patients with unilateral coronal craniosynostosis, this study was a double-blinded multi-institutional cohort study between patients treated at the Yale School of Medicine and the Children's Hospital of Pennsylvania (CHOP). Patients who had radiographic confirmation of non-syndromic unilateral coronal craniosynostosis and received cranial vault remodeling in infancy were included in the study. Patients were at an age of cranial maturity, 8.0 years of age or older, at time of testing. All patients were administered the Weschler Fundamentals (WF), Weschler Abbreviated Scale of Intelligence (WASI), Beery VisuoMotor Integration (VMI), Behavior

Rating Inventory and Executive Function (BRIEF), Child Behavior Checklist (CBCL), demographic survey, Youth Quality of Life (YQOL), and 3D photograph.

Metopic Craniosynostosis Categorization

All patients with radiographic confirmation of metopic craniosynostosis and a history of cranial vault remodeling in infancy were recruited from the Yale School of Medicine. All patients were school age, 6.0 years or older, at the time of testing. All patients were administered the WF, WASI, Beery VMI, BRIEF, Behavior Assessment System for Children (BASC), and demographic survey.

SMAD6 Comparison

This was a prospective double-blinded cohort study conducted at the Yale School of Medicine. Subjects were included if they were diagnosed with midline non-syndromic craniosynostosis and received surgical correction at an earlier age. School age patients currently 6.0 years of age or older were included.

Patients with *SMAD6* mutations were identified from the index study.[57] Non *SMAD6* controls who were diagnosed with midline craniosynostosis and underwent whole exome sequencing found to have wild type *SMAD6* alleles were included. Controls were matched by current age (within one year), gender, race, synostosis type, and surgery type (whole vault cranioplasty or strip craniectomy).

All tests were administered by a single blinded tester between June 2017 – April 2018. Test subjects were blinded as to the testing groups under scrutiny. Subjects in the index

study were recruited nationwide via social media. In order to keep a standardized test administrator, subjects who were unable to travel due to geographical constraints were able to participate in virtual webcam testing. Mountable Logitech C615 webcams (Logitech, Lausanne Switzerland) and testing materials were sent to participant homes, which allowed the administrator to interact with and watch participants complete tasks in real time. All patients were administered the WF, WASI, Beery VMI, BRIEF, BASC2, and demographic survey.

Functional MRI Analysis

Surgically corrected adolescent patients age ≥9 with isolated nonsyndromic metopic or unilateral coronal synostosis operated and treated by the senior author were recruited. Age/gender/handedness non-craniosynostosis healthy matched controls were recruited from the Yale Child Studies Center.

Prior to fMRI scan, each craniosynostosis subject was administered the Wechsler Intelligence Scale for Children 3rd edition (WISC-III) and all subject guardians were given the BRIEF survey.

Event Related Potential Analysis

Craniosynostosis infants were recruited at the Yale Craniofacial Clinic by our lab (Jenny F. Yang, MD; Roberto Travieso, MD; Joel Beckett, MD) if they were diagnosed with non-syndromic craniosynostosis and were planned for surgical correction. Patients were tested pre-operatively with both functional and event-related potentials and then returned for the same testing battery three months post-operatively.

Once these same patients were 6.0 years of age or older, they were recruited for follow up neurocognitive testing. Due to particular sensitivity to reading/language delays, all patients were administered the WF, WASI, and demographic survey.

Neuropsychiatric Testing Battery

All neuropsychiatric testing for the SMAD6 and event related potential study arms were performed by the same tester (R.W.). All neurocognitive testing in the remainder study arms was administered between R.W., K.G., A.S., P.A., and J.N.. All test administrators were blinded to the clinical variables, synostosis side, and patient demographics. Surveys were administered to the parent or legal guardian of the participant to gauge behavior, psychological functioning, and record demographic data.

Neurocognitive Tests

The neurocognitive assessment paradigm utilized is outlined below using previously published techniques for patients with craniosynostosis.[25,30]

1. Weschler's Abbreviated Scale of Intelligence (WASI)

The WASI is an individually administered assessment which is designed to measure performance, verbal, and full-scale intelligence quotient (IQ). Verbal IQ is determined by subtests in vocabulary and word similarities which quantify the patient's word knowledge and verbal reasoning. Performance IQ is quantified based on subtests in

block design and matrix reasoning which quantify visuospatial reasoning and the ability to separate figure from ground in visual stimuli.[74]

2. Weschler's Fundamentals (WF)

The WF is an individually administered assessment designed to provide a global assessment of age-based academic achievement. The verbal component consists of domain scores for word reading, reading comprehension, and reading composite. The spelling section asks the child to write dictated letters and words. The mathematical component assesses the patient's ability to perform multiple arithmetic calculations in a limited time period.

3. Beery-Buktenica Developmental Test of Visuo-Motor Integration (Beery VMI)

The fifth edition of the Beery VMI is an individually administered assessment which quantifies the patient's ability to integrate visual stimuli and motor responses. The child must by draw geometric forms (VMI), visually distinguishing between similar items (Visual Perception), and perform fine hand and finger movements (Motor Coordination).

4. Wechsler Intelligence Scale for Children 3rd edition (WISC-III)

Similar to the WASI, the WISC-III generates a Full Scale IQ that represents a child's general intellectual ability. It also provides five primary index scores: Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory

Index, and Processing Speed Index. These indices represent a child's abilities in discrete cognitive domains.

Parental/Guardian Surveys

1. Behavior Rating Inventory of Executive Function (BRIEF)

The BRIEF uses parent questionnaires to assess executive functioning in the home and school surroundings.7 Results are summarized with eight subcategories: Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials, Monitor, Behavioral Regulation, Metacognition, Global Executive Composite.

2. Behavior Assessment System for Children (BASC), Second Edition

The BASC is administered in a questionnaire format that lists numerous aspects of behavior and personality functioning. Results are summarized with four subcategories.

3. Child Behavior Checklist (CBCL).

The CBCL is administered in a questionnaire format that assesses behavior and mental health functioning. Results are summarized with four subcategories: Competence (Activities + Social + School), Internalizing Problems (Anxious/Depressed + Withdrawn/Depressed), Externalizing Problems (Rule-breaking + Aggressive behavior), Total (Internalizing + Externalizing). Category T scores above 70 (98^{th} percentile) represent behaviors in the range of clinical concern.

4. Demographic Survey

Twenty-two socioeconomic and demographic factors significant to neuropsychiatric development were collected[75-79].

Quality of Life Survey

The Youth Quality of Life with Facial Differences (Seattle Life Group, Seattle WA) is an individually administered patient reported outcome measure. The questionnaire quantifies domain scores in positive and negative consequences of the disease process, negative self-image, positive self-image, stigma, and coping resulting from the patients facial asymmetry.[80]

Computed Tomographic Scan Analysis

Pre-operative CT scans for patients with metopic craniosynostosis were analyzed using Mimics software (Materialise, Leuven, Belgium). After orienting each image along the Frankfort horizontal plane, the endocranial bifrontal angle (EBA) was measured from the endocranial midline to the lateral portion of the orbital rim at the same axial plane in line with the posterolateral aspect of the lateral orbital rim.[12,48,81] (Figure 1) Inter-rater reliability was evaluated by independent measurements by different individuals blinded to the academic and cognitive outcomes. Neurocognitive outcomes were sorted by radiographic severity of metopic craniosynostosis (endocranial bifrontal angle less than or greater than 124°).[12,48]

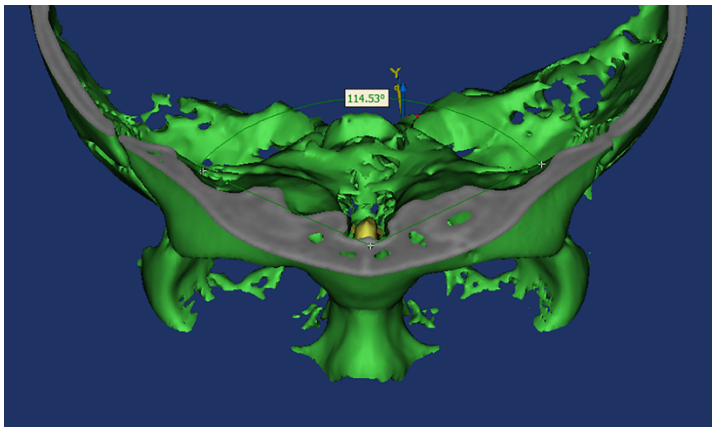

Figure 1. Radiographic assessment of endocranial bi-frontal angle for categorizing metopic craniosynostosis severity.

Direct Neuroimaging and Genetic Analysis

Functional MRI

All fMRI testing was administered by J.Y and R.W.. The frustration-induction Go-NoGo task was a mixed blocked/event-related fMRI design. The task was presented using E-prime software (Sharpsburg, PA; Figure 2). Subjects were instructed to view a steady stream of common objects (balls, hats, chairs, etc.) and to press a button for every object in a green frame (Go), but to inhibit response when an object appears in a red frame (NoGo). Initially, subjects were told that they would earn points that can be exchanged for a prize. Incorrect, omitted, or late responses resulted in a large X on the screen and a buzzer indicating a failure to gain points (negative feedback). Every 20 trials, the number of accumulated points appeared on the screen.

	Block 1 - Winning	Block 2 - Losing	Block 3 - Recovery
Stimulus Presentation	800-1150 ms	600-950 ms	800-1150 ms
Correct No-Go Response	-20 ms	-50 ms	-20 ms
Incorrect No-Go Response	+70 ms	+50 ms	+70ms
Points for Go Trial Correct/Incorrect	+3/0	0/-15	+15/+1
Points for No-Go Trial Correct/Incorrect	+7/0	0/-15	+25/+1

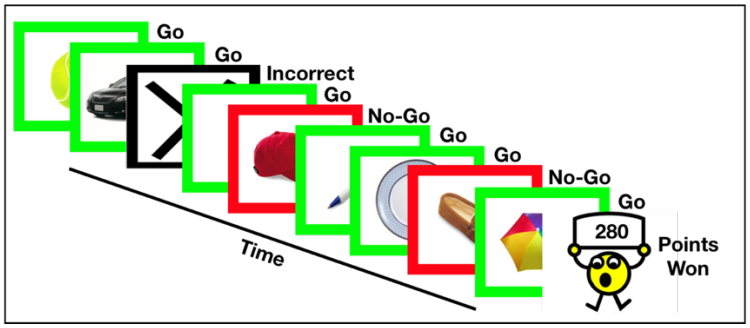

Figure 2. Frustration-induction Go-No/Go task. Upper panel shows latency windows for stimulus presentation and correct responses by 3 task blocks. Lower panel shows a sample of the task stimuli.

Unbeknownst to the subject, the NoGo error rate was maintained at 50±10% by adjusting stimulus duration. Stimulus duration was increased with each NoGo error and decreased with each correct response. This was intended to provide the same level of challenge for all subjects and to obtain a sufficient number of correct NoGo trials.

Also unbeknownst to the subject, the task contained three conditions designed to induce frustration and require emotion regulation. In the first block called "win", participants saw their points steadily increase to >1000. In the second block called "lose", changes in the point-adjustment algorithm caused the task to become more difficult, thereby leading to a loss of all of accumulated points (induction of frustration). The last block called

"recovery" had a return to the more generous algorithm, subjects regained their points and, ultimately, always won their desired prize.

Simple GoNogo tasks, defined by the use of identical stimuli, most often activate the inferior occipital gyrus, fusiform gyrus, posterior cerebellum, superior medial wall, and precuneus.[82]

Task performance was evaluated by average stimulus time. Given the set error rate (more incorrect answers leads to increased stimulus times), average stimulus interval was compared with two tailed T-tests. All statistical analyses were done using SPSS statistical software (IBM Corporation, USA).

Event Related Potentials

All initial event related potentials were conducted by J.Y., R.T., C.C, A.S., R.W., and T.H.. At each visit participants were first administered the BSID by a licensed child psychologist. Immediately following, they participated in ERP testing. Infants were presented with a non-native phoneme discrimination paradigm involving the Hindi retroflex phoneme /da/ and the dental phoneme /da/. Auditory stimuli were set at 80 dB, and EEG was recorded at 250 Hz with a 128-channel HydroCel Geodesic Sensor Net. The complete paradigm stimulus time was approximately five minutes.

The MMN component was calculated by subtracting the dental wave from the retroflex wave. All responses following each stimulus were grand averaged (Figure 3). The MMN

was taken as the largest negative amplitude in the difference wave between 80-300ms after stimulus presentation.

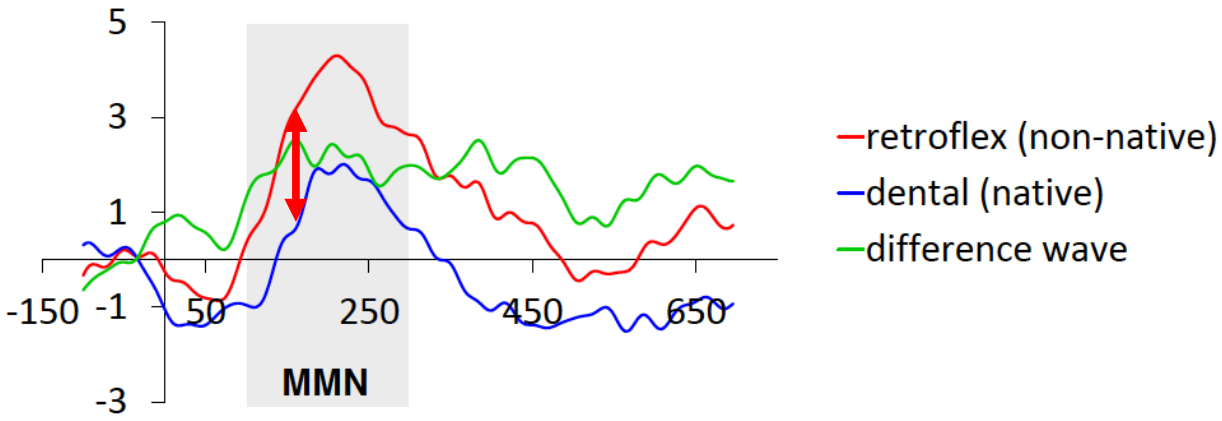

Figure 3. Sample output and calculation of the MMN difference wave taken between the brain waves in response to the native dental phonemes and non-native retroflex phonemes.

Genetic Analysis

All genetic analysis was performed by author A.T.. Whole exome sequencing was performed of the case-parent trios of interest using DNA prepared from buccal swab samples according to standard protocols. Exome capture was performed using the IDT xGen capture reagent, which was followed by 99 base paired-end sequencing on the Illumina HiSeq 2000 instrument. Sequence reads were aligned to the GRCh37/hg19 human reference genome using BWA-Mem. Local realignment and quality score recalibration were performed using the GATK pipeline, after which variants were called using the GATK Haplotype Caller. A Bayesian algorithm, TrioDeNovo, was used to call de novo mutations. VQSR 'PASS' variants with an ExAC allele frequency

$\leq 10^{-3}$ sequenced to a depth of eight or greater in the proband and 10 or greater in each parent with Phred-scaled genotype likelihood scores >30 and de novo quality scores ($\log_{10}$(Bayes factor)) >6 were considered. Independent aligned reads at variant positions were visualized in silico to remove false calls. All retained calls had de novo genotype quality scores of 100. Transmitted variants were called as per above, and all variants were annotated using ANNOVAR with allele frequencies assigned to each variant from the ExAC database.

Statistical Analysis

All statistical analysis unless otherwise specified was performed with SPSS Statistics Version 25 (IBM®).

Unilateral Coronal Craniosynostosis Neuropsychiatric Outcomes

The Yale and CHOP cohorts were compared using t-tests for continuous variables and Fisher's exact contingency table for categorical values. Correlations were calculated between individual test scores and continuous patient variables, with a strong correlation coefficient (r) defined as $\geq |0.7|$. Head-to-head comparison of test scores between binary variables (i.e. right and left ULC, male and female, and breast-feeding status) were performed using unpaired two-tailed t-tests. Significant values were adjusted for confounding variables using multivariate linear regressions. $P<0.05$ was set

as significant for all tests. Post hoc power analysis was tabulated using G*power (Universität Düsseldorf).

Metopic Craniosynostosis Neurocognitive Comparison to Severity

Frequency statistics were used to report demographics and neurocognitive outcomes. Independent students t-tests were used to compare continuous variables between the two groups of metopic radiographic severity. Pearson's bivariate correlation evaluated demographic variables' impact on IQ and academic achievement. Significant demographic variables were included in a linear regression model. Academic performance results were controlled for full-scale IQ and age at surgery. IQ was controlled for maternal education, paternal education and income. Statistical significance defined as $p < 0.05$.

SMAD6 Comparison to non-SMAD6 Neurocognitive Outcomes

Head to head comparison of test scores between *SMAD6* nonsyndromic craniosynostosis patients and non-*SMAD6* matched controls were performed using unpaired two-tailed t-tests. Differences between demographic/socioeconomic factors were calculated using t-tests for continuous variables and Fisher's exact contingency table for categorical values. Correlations were calculated between each individual test score and each demographic variables, with a strong correlation coefficient (r) defined as $\geq |0.7|$. Follow-up multiple regressions were performed to control for demographic factors that correlated significantly with cognitive scores. Post-hoc power analysis was

performed with G*Power (Universität Düsseldorf, Düsseldorf Germany). $P<0.05$ was set as significant for all tests.

fMRI Comparison

fMRI data was gathered with a 3T Siemens TIM Trio scanner. The functional data were motion corrected and time sliced suing Matlab (Mathworks). Within group whole-brain T-tests were conducted using BioImage Suite with a cluster threshold of 675 voxels and $p<0.05$. T-maps were created between desired task conditions and non-task conditions including resting state. Resultant T-maps were then used to conduct T-tests between test subjects and controls. Seed-based analysis using region of interest (ROI) identified from between-group T-tests were performed. Anatomical landmarks were corroborated with a clinical neuroradiologist.

For inter-task and intra-brain comparison, anatomical ROIs were defined based on significant regions found with seed-based analysis. Averaged blood-oxygenation-level-dependent (BOLD) contrast was calculated within the defined regions per subject. Statistical analyses were performed using paired and independent sample T-tests.

ERP and BSID Comparison with Neurocognitive Outcomes

Comparison of ERP/BSID with future neurocognitive performance was assessed with correlations. Strong correlation was set at $R=0.70$ and $p<0.05$ was significant.

Results

Unilateral Coronal Craniosynostosis Neurodevelopmental Outcomes

Subjects

A total of 20 patients (12 Yale, 8 CHOP) successfully participated in the study (Table 1). There were no significant differences in demographic, clinical, or testing results between the two institutions.

Demographics	Unilateral Coronal
n	20
Sex (n)	
Male	9 (45%)
Female	11 (55%)
Age at Surgery (days)	246.6 ± 5.0
Operation Type (n)	
Cranial Vault Remodeling with Frontal Orbital Advancement	20 (100%)
Synostosis Side (n)	
Right	10 (50%)
Left	10 (50%)
Age at Testing (years)	12.1 ± 0.2
Race (n)	
White	13 (65%)
Hispanic	2 (10%)
Black	2 (10%)
Other	3 (15%)
Gestational Age (weeks)	37.7 ± 0.2
Birth Weight (ounces)	107.4 ± 1.5
History of Breast Feeding (n)	14 (70%)
Siblings with Craniosynostosis (n)	0
Family with Craniosynostosis (n)	1 (5%)
Primary Language (n)	
English	19 (95%)

Spanish	1 (5%)
Homeschooled (n)	0 (0%)
Grade at Time of Testing	6.1 ± 0.2
Parental Age at Birth (Years)	
Mother	32.0 ± 0.2
Father	35.0 ± 0.2
Marital Status (n)	
Married	19 (95%)
Divorced	1 (5%)
Maternal Education (n)	
High School	4 (20%)
Trade School	1 (5%)
College	11 (55%)
Grad School	4 (20%)
Paternal Education (n)	
High School	8 (40%)
Trade School	4 (20%)
College	6 (30%)
Grad School	2 (10%)
Household Income	
<$25,000	2 (10%)
$25-50,000	2 (10%)
$50-75,000	7 (35%)
$75-100,000	5 (25%)
$100-250,000	3 (15%)

Table 1. Demographic and socioeconomic variables of the 20 unilateral coronal patients.

The average age at testing was 12.1 ± 0.2 years and 55% of patients were female (Table 1). White patients comprised 65%, Hispanic 10%, Black 10%, and "other" 15%. Patients were evenly split among right and left ULC diagnosis. The average at surgery was 246.6 ± 5.0 days with all patients having undergone cranial vault remodeling with frontal orbital advancement (100%).

Mean gestational age was 37.7 ± 0.2 weeks and average birth weight was 107.4 ± 1.5 ounces. Seventy percent of patients had a history of breast feeding while the remaining

30% were exclusively formula fed. The most common income category range was $50-75,000 (35%). Highest maternal education was most frequently college (55%), and highest paternal education was most commonly high school (40%).

Neurocognitive Test Performance

ULC patients on average achieved academic performance percentiles above the national mean (word reading 76.3%, reading comprehension 60.8%, reading composite 68.1%, spelling 61.4%) with the notable exception of numerical operations (47.2%; Table 2). While all language based scores were higher, word reading was statistically higher than numerical operations (p=0.022).

The average Verbal IQ (VIQ) was 117.3, Performance IQ (PIQ) was 106.4, and Full-Scale IQ (FSIQ) was 112.5. Seventy-five percent of patients had higher VIQ than PIQ, which approached, but did not reach statistical difference (p=0.052).

Patients on average had percentile scores below the national mean on all Beery-Buktenica Developmental Tests (visual-motor integration [VMI] 42.5%, visual perception 49.6%, motor coordination 26.0%). Inter-test comparison revealed motor coordination scores were significantly poorer than both VMI and visual perception scores (p=0.027, p=0.005).

Neurocognitive Test	Score	SD	Percentile
Weschler Fundamentals: Academic Skills			
Word Reading	113.3	17.2	76.3
Reading Comp	101.1	21.7	60.8
Reading Composite	107.7	19.2	68.1
Spelling	105.8	21.1	61.4
Numerical Operations	98.1	22.7	47.2
Weschler Abbreviated Scale of Intelligence			
Verbal IQ	117.3	18.8	76.9
Performance IQ	106.4	16.4	59.7
Full IQ	112.5	17.1	70.7
Beery-Buktenica Developmental Tests			
Visual-Motor Integration	96.5	12.4	42.5
Visual Perception	99.5	12.1	49.6
Motor Coordination	85.7	16.8	26

Behavioral Survey	Score	SD	Percentile
Behavior Rating Inventory of Executive Function			
Inhibit	47.0	5.7	51.4
Shift	46.2	7.6	46.2
Emotional Control	45.7	10.7	39.4
Initiate	49.6	11.6	51.6
Working Memory	49.9	10.3	55.5
Plan/Organize	50.1	11.5	51.5
Organization of Materials	49.8	8.6	53.7
Monitor	47.1	8.8	46.8
Behavioral Regulation Index	45.6	8.3	38.7
Metacognition Index	49.3	9.9	48.9
Global Executive Composite	52.1	8.2	57.4
Child Behavior Checklist*			
Competence	48.3	11.1	42.4
Internalizing	49.0	12.1	46.2
Externalizing	45.1	12.0	31.5
Total	45.3	14.8	32.0

*Child Behavior Checklist scores range from the normal spectrum of non-clinically referred children (30-70) to clinical behavior ranges (>70). Increasing scores represent increasing behavioral issues.

Table 2. Neurocognitive test and parental/guardian behavioral survey results. Scores listed in averages. IQ values normalized to 100.

Behavioral Survey Performance

ULC subjects on average scored within one standard deviation of the mean on all parental/guardian behavior assessments (Table 2). Patients performed the poorest on behavioral regulation (38.7%) and emotional control (39.4%) in the Behavior Rating

Inventory of Executive Function survey. No patient scored in the range of clinical concern (T-score >70) on any of the Child Behavior Checklist (CBCL) assessments.

Impact of Patient Factors on Neurocognitive Performance

Correlations between all string variables and neurocognitive tests yielded moderate correlations between increasing paternal education and improved visual perception (r=0.450, p=0.046; Table 3), increasing household income with increasing VIQ (r=0.628, p=0.004), and decreasing birth weight with increasing numerical operations scores (r=-0.58, p=0.015).

	Score	SD	Percentile	p-value
Spelling				
RULC	116.8	13.3	81.8	0.033*
LULC	94.8	22.4	41.1	
Motor Coordination				
Surgery <7 month	91.9	15.7	33.9	0.067
Surgery >7 months	78.1	15.7	16.4	
Male	76.1	18.6	12.3	0.024
Female	93.5	11.8	37.3	
VMI				
Exclusively Formula Fed	85.4	7.6	21	0.014
History of Breast Feeding	100.8	11.7	49.5	
Visual Perception				
Exclusively Formula Fed	89.2	13.4	28.8	0.031
History of Breast Feeding	102.8	10.2	56.5	
Performance IQ				
Exclusively Formula Fed	93.8	9.7	36.6	0.034
History of Breast Feeding	111.5	16.5	68.9	

	Pearson Correlation	p-value
Household Income vs Verbal IQ	0.628	0.004
Paternal Education vs Visual Perception	0.450	0.046
Birth Weight vs Numerical Operations	-0.578	0.015

*Adjusted p-value controlling for performance IQ, verbal IQ, full-score IQ, age at surgery, sex, breast-feeding, paternal education, household incom

Table 3. Comparison of test scores to patient variables. Two tailed unpaired t-tests were performed for binary variables and Pearson correlations were performed for continuous. $P<0.05$ was significant, $r \geq 0.7$ was a strong correlation.

Patients operated prior to 7 months performed better on motor coordination than those operated after 7 months, approaching but not reaching statistical significance (33.9% vs 16.4%; p=0.067). Females had significantly better VMI than males (93.6% vs 76.1%; p=0.024). Patients that were breast-fed for any amount of time had higher performance IQ (110.6 vs 93.8; p=0.034), VMI (49.5% vs 21.0%; p=0.014), visual perception (56.5% vs 28.8%; p=0.031), and trended towards better performance in all categories (Figure 4).

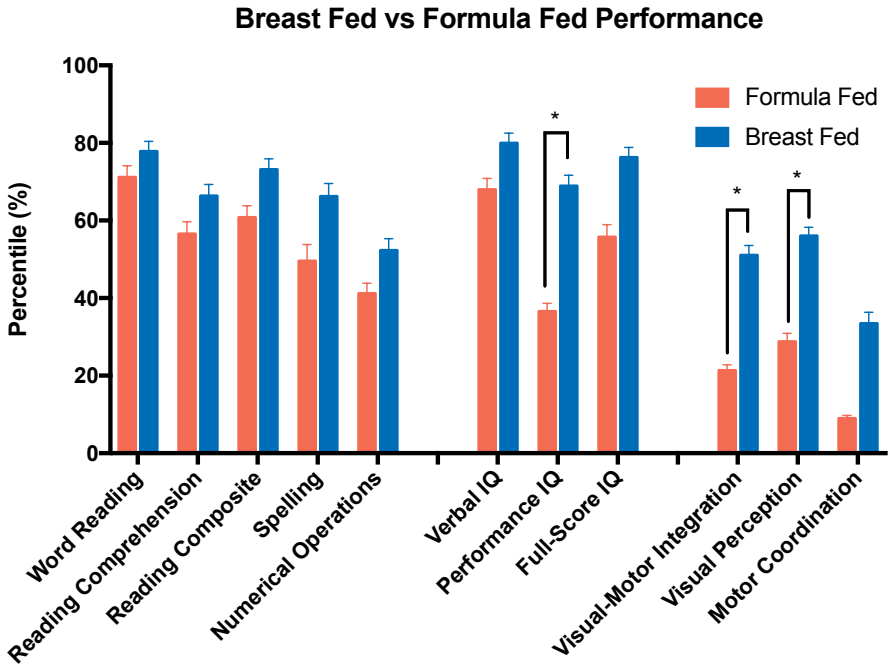

Figure 4. Unilateral coronal subjects with any breast feeding in infancy had improved Verbal IQ, visual-motor integration, and visual perception. *p<0.05.

Head-to-head comparison found significantly higher spelling scores for right ULC compared to left (81.8% vs 41.1%; p=0.017; Figure 5). While no significant differences

were found for any other neurocognitive or behavioral score, subjects with right sided fusion scored higher on all language/verbal tests. Follow-up multiple regression between coronal sidedness and spelling scores was performed to control for all three IQ measures (VIQ, PIQ, FSIQ) and any variables that significantly impacted performance on any tests (age at surgery, sex, breast-feeding status, paternal education, household income, age at testing, and race). Adjusted analysis revealed right-sided ULC still significantly predicts higher spelling scores (R^2 0.650, p=0.033).

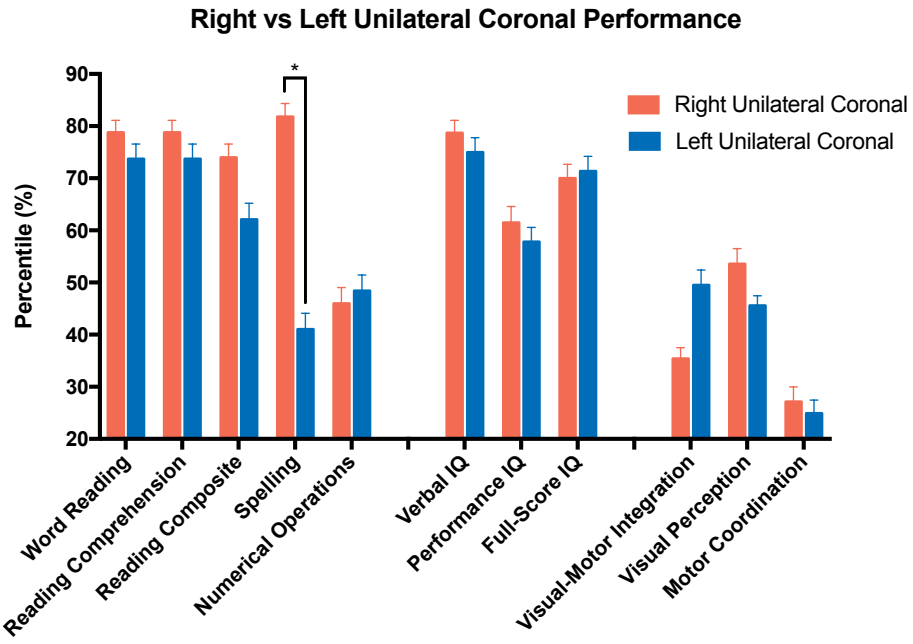

Figure 5. Right unilateral coronal (ULC) subjects performed significantly better than left ULC patients in spelling. *p<0.05

Post-hoc power

Post-hoc power analysis (α=0.05) for all significant correlation, t-test, and multivariate regression values yielded a power ranging from 77.5% - 96.5%, with exception to the

correlation between paternal education and visual perception, which was moderately powered at 69.9%.

Metopic Craniosynostosis Neurocognitive Outcomes

Subjects

Twenty patients met inclusion criteria and were predominantly male (90%) with a mean age at time of testing was 10.2 years (6.3–14.6 years). Mean age at the time of surgery was 11.0 months (3.2–34.8 months). Complete demographics are available in Table 4.

Patient Characteristics		
n		20
Gender		90% (18) Male
		10% (2) Female
Age at Surgery		0.92 ± 0.8 Years
Age at Testing		10.2 (±2.3) Years
Race	White	63.2%
	Hispanic	10.5%
	Black	10.5%
	Other	15.8%
Gestational Age		37.2 (±3.5) Weeks
Birth Weight		6.22 (±1.9) lb.
Breast Feeding		55%
Sibling w Head Irreg.		25%
Language	English	85%
	Spanish	5%
	Other	10%
Marital Status	Married	84.2%
	Divorced	15.7%
Paternal Age	Mother	30.9 ± 7.4
	Father	30.8 ± 9.5
Maternal Education	Highschool	21.1%
	Some College / Tech	21.1%
	College Grad	36.8%
	Graduate Degree	21.1%
Paternal Education	Highschool	22.2%
	Some College / Tech	33.3%
	College Grad	27.8%
	Graduate Degree	16.7%
Income	< 25 K	5.5%
	25-65 K	38.9%
	> 65 K	55.6%

Table 4. Demographics of metopic craniosynostosis patient population.

Neurocognitive Test Performance

Among the 20 cranially mature patients tested, mean verbal IQ was 114.2 ± 15.8 (70.9% percentile), performance IQ was 107.8 ± 12.7 (66.1% percentile), and full-scale IQ was 111.7 ± 13.1 (63.1% percentile; Table 4)

Academic achievement was slightly above national averages in word reading (53.9%), reading comprehension (53.4%), reading composite (53.5%), and numerical operations (52.9%). However, patients scored below the national mean for spelling (44%; Table 5).

Neurocognitive Results Domain	Score	%
Verbal IQ	114.2 ± 15.8	70.9
Performance IQ	107.8 ± 12.7	66.1
Full Scale IQ	111.7 ± 13.1	63.1
Word Reading	101.1 ± 10.4	53.9
Read Comprehension	101.6 ± 10.0	53.4
Reading Composite	102.0 ± 9.0	53.5
Spelling	97.3 ± 12.1	44
Numeric Operations	102.5 ± 17.4	52.9
Visual-Motor Integration	93.5 ± 10.3	33.9
Visual Perception	98.3 ± 13.6	45.3
Motor Coordination	85.1 ± 15.4	23.5

Table 2. Neurocognitive outcomes of metopic craniosynostosis patients.

With respect to the Berry-Buktenica Developmental tests, visuo-motor integration [VMI], visual perception and motor coordination were all below national means at 33.9%, 45.3%, and 23.5% percentiles, respectively.

Analysis of Severity

Patients were grouped into "moderate" (endocranial bifrontal angle >124°) and "severe" (endocranial bifrontal angle <124°). Inter-rater reliability was high (Average Difference: 0.89°; p = 0.089). Thirty-six percent of patients had moderate metopic synostosis with a mean angle of 126.9° ± 2.1 while 64% had severe metopic craniosynostosis with a mean angle of 119.3° ± 5.2.

Patients with severe metopic craniosynostosis had significantly lower academic achievement scores in both word reading (95.3 vs. 113; p = 0.035) and reading composite (98.3 vs. 109.5; p=0.014). The severe cohort performed in reading comprehension (101.3 vs. 105.0; p = 0.448), numerical operations (96.7 vs. 116.5; p = 0.064) and spelling (90.2 vs. 110.0; p = 0.149), but the results were not statistically significant. (Table 6)

With respect to IQ, severe metopic craniosynostosis patients had lower verbal, performance and full-scale IQs, but the results did not achieve significance (Table 6).

Beery-Buktenica developmental tests were not statistically different for VMI (88.5 vs. 98.5; p = 0.324), visual perception (93.8 vs. 111.25; p = 0.416), or motor coordination (85.7 vs. 81.3; p = 0.324; Table 6).

Analysis by Severity	Moderate (36%) Mean	Severe (64%) Mean	p Value
Endocranial Bifrontal	126.9° ± 2.1	119.3° ± 5.2	**0.008**
Word Reading	113 ± 8.3	95.3 ± 9.6	**0.035**
Read Comprehension	105 ± 0.8	101.3 ± 7.8	0.448
Reading Composite	109.5 ± 4.7	98.3 ± 7.7	**0.014**
Spelling	110 ± 14.0	90.2 ± 10.0	0.149
Numeric Operations	116.5 ± 12.4	96.7 ± 12.1	0.064
Verbal I.Q.	121 ± 15.7	114.8 ± 7.9	0.398
Performance I.Q.	116.5 ± 13.3	114 ± 12.1	0.901
Full Scale I.Q.	121 ± 11.3	115.4 ± 7.6	0.501
Visual-Motor Integration	98.5 ± 15.9	88.5 ± 9.9	0.324
Visual Perception	111.25 ± 14.7	93.8 ± 11.1	0.416
Motor Coordination	81.25 ± 21.5	85.7 ± 17.3	0.324

Table 3. Neurocognitive outcomes stratified by pre-operative severity of metopic craniosynostosis.

Sagittal and Metopic SMAD6 Neurocognitive Outcomes

Subjects

Among 26 subjects identified nationwide with the *SMAD6* mutation, 10 met inclusion criteria and none were excluded. All *SMAD6* mutations were rare (frequency <2 x 10-5) loss of function mutations. Nearly all were absent in the ExAC database, which contains over 120,000 alleles, and 7 of the 10 studied subject also harbored at least one copy of the common *BMP2* risk allele.[57] All 10 subjects participated in our study (average age 10.1 years; 9 male, 1 female; 6 White, 2 Hispanic, 1 Black, 1 Other; 8 metopic, 2 sagittal; 9 received whole vault cranioplasty, 1 received strip craniectomy; Table 7). Subjects were matched according to age, gender, race, synostosis type, and operation type to 10 non-*SMAD6* non-syndromic craniosynostosis controls (average age

9.8 years). Among 11 controls tested, one was excluded for a new seizure disorder diagnosis.

Demographics	SMAD6 synostosis	Non-SMAD6 synostosis	p-value	
Age	10.1 Years	9.8 Years	0.809	Matched Variables
Sex	1 Female	1 Female	1.000	
Race	6 White, 2 Hispanic, 1 Black, 1 Other	6 White, 2 Hispanic, 1 Black, 1 Other	1.000	
Synostosis Type	8 Metopic; 2 Sagittal	8 Metopic; 2 Sagittal	1.000	
Operation	9 CVR; 1 Strip	9 CVR; 1 Strip	1.000	
Age at Surgery	360	357	0.985	
Gestational Age	36.7 weeks	38.2 weeks	0.301	
Birth Weight	102.1 Oz	111.4 Oz	0.516	
Breast Feeding	2.9 months	3.1 months	0.955	
Sibling with CSC	3 pt	1 pt	0.287	
Other Family Hx of CSC	1 pt	1 pt	1.000	
Primary Languages at home	2 English/Spanish; 8 English	1 English/Spanish; 1 English/Portuguese; 8 English	1.000	
Home Schooling	0	0	1.000	
Mother's Age at Birth	31.6 years	30.1 years	0.609	
Father's Age at Birth	33.2 years	32.7 years	0.841	
Parental Marital Status	2 Divorced	2 Divorced	1.000	
Maternal Education	2.4	2.3	0.905	
Paternal Education	2.1	2.4	0.489	
Parental Education	4.5	4.3	0.846	
Household Income	7 $25K-$65K/year; 3 >$65K	3 $25K-$65K/year; 7 >$65K	0.081	

Table 7. No significant difference between any demographic or socioeconomic variables. Demographic and socioeconomic variables gathered from survey and chart review. P-values calculated using Fischer's exact and two-tailed T-tests. Non-SMAD6 nonsyndromic craniosynostosis were matched using all variables in blue to SMAD6 nonsyndromic craniosynostosis.

Among 20 demographic and socioeconomic variables collected, there were no significant differences between cases and controls (Table 7). All patient had obtained good aesthetic results in terms of symmetry and shape at the time of testing, and no patient was pursuing further correction.

Head-to-head T-test comparison between SMAD6 and non-SMAD6 controls

SMAD6 nonsyndromic craniosynostosis patients performed significantly worse than non-*SMAD6* synostosis patients on numerical operations (36.7% vs 68.5%; p=0.012), Performance IQ (PIQ; 47.8% vs 83.5%; p=0.004), Full Score IQ (FSIQ; 54.1% vs 88.8%; p=0.007), and motor coordination (7.8% vs 36.2%; p=0.007; Table 8). *SMAD6* synostosis patients trended towards worse performance on word reading (p=0.077), reading composite (p=0.062), and Verbal IQ (VIQ; p=0.057), but this did not achieve significance.

Neurocognitive Test	Univariate T-Test			Multivariate Regression						
	Percentile (%)			Coefficient p-value						
	SMAD6	Control	P-value	SMAD6 vs Control	Age	Age at Surgery	Parental Education	Household Income	β	Constant p-value
Word Reading	48.4	65.3	**0.077**	0.212	0.110	0.549	0.900	0.734	19.896	<0.001
Reading Comp	52.9	60.3	0.250	0.403	0.137	0.733	0.403	0.587	12.518	<0.001
Reading Composite	49.0	64.6	**0.062**	0.158	0.050	0.737	0.718	0.650	13.309	<0.001
Spelling	37.0	54.2	0.185	0.206	0.020	0.195	0.337	0.844	19.720	<0.001
Numerical Operations	36.7	68.5	**0.012***	**0.046***	0.077	0.706	0.882	0.785	23.265	<0.001
Verbal IQ	55.9	86.7	**0.057**	**0.061**	0.296	0.564	0.207	0.981	30.972	<0.001
Performance IQ	47.8	83.5	**0.004***	**0.018***	0.060	0.439	0.948	0.362	28.799	<0.001
Full IQ	54.1	88.8	**0.007***	**0.010***	0.070	0.396	0.421	0.564	47.128	<0.001
Visuo-Motor Integration	27.9	42.0	0.191	0.143	0.088	0.462	0.877	0.223	21.044	<0.001
Visual Perception	37.1	51.2	0.250	0.270	0.198	0.085	0.418	0.965	22.008	<0.001
Motor Coordination	7.8	36.2	**0.007***	**0.043***	0.952	0.008	0.187	0.405	23.269	0.001

Table 8. Head to head univariate T-test between SMAD6 and non-SMAD6 controls with follow up multivariate regression controlling for age at testing, age at surgery, parental education, household income.

Correlation Analysis

Follow up correlation analysis was undertaken to establish the influence of patient factors on neurocognitive performance. Among individual correlations between all 20 demographic factors and all 11 neurocognitive test scores, only younger age at testing, earlier age at surgery, increased parental education, and increased household income significantly correlated with improved neurocognitive test scores (Table 9). These four factors contributed to 17 significant correlations, none of which were strong ($p<0.05$, $r<|0.6|$).

		WR	RC	WR+RC	Spell	Num. Oper.	VIQ	PIQ	FSIQ	VMI	VP	MC
Age at Testing	**Pearson Correlation**	-0.383	-0.441	-0.490	-0.513	-0.421	-0.329	-0.470	-0.447	-0.479	-0.419	-0.582
	p-value	0.096	0.052	0.026	0.021	0.065	0.156	0.036	0.048	0.033	0.066	0.007
Age at Surgery	**Pearson Correlation**	0.076	-0.428	-0.154	0.028	-0.062	-0.481	-0.333	-0.450	-0.407	-0.425	-0.319
	p-value	0.749	0.060	0.517	0.907	0.796	0.032	0.151	0.046	0.075	0.062	0.170
Parental Education	**Pearson Correlation**	-0.077	0.471	0.176	0.102	0.050	0.513	0.245	0.417	0.373	0.090	0.396
	p-value	0.748	0.036	0.459	0.670	0.835	0.021	0.299	0.067	0.105	0.705	0.084
Household income	**Pearson Correlation**	0.366	0.504	0.521	0.489	0.424	0.474	0.499	0.553	0.597	0.067	0.327
	p-value	0.135	0.033	0.027	0.040	0.080	0.047	0.035	0.017	0.009	0.792	0.186

Table 9. Significant correlations between neurocognitive scores and patient factors. All significant ($p<0.05$) correlations highlighted in blue. WR = word reading. RC = reading comprehension. WR+RC = reading composite. Spell = spelling. Num. Oper. = numerical operations. VIQ = verbal IQ. PIQ = performance IQ. FSIQ = full-scale IQ. VMI = visuomotor integration. VP = visual perception. MC = motor coordination.

Controlling for significant patient factors

Multiple regressions were undertaken between *SMAD6* status and neurocognitive test scores to control for the four significant correlating factors (age at testing, age at surgery, parental education, and household income).

Analysis revealed that even after controlling for contributing patient factors, *SMAD6* mutation status significantly predicted lower scores on numerical operations (p=0.046), PIQ (p=0.018), FSIQ (p=0.010), and motor coordination (p=0.043; Table 8). VIQ trended towards significance (p=0.061).

Parental Surveys

On the BRIEF survey, *SMAD6* cases scored significantly worse on inhibition (p=0.003) and behavior regulation (p=0.032).

On the BASC-2 survey, *SMAD6* cases had significantly worse hyperactivity (p=0.007), aggression (p=0.008), conduct problems (p=0.029), social skills (p=0.039), and functional communication (p=0.018).

Power Analysis

Post hoc power analysis was conducted for all significant test values. Comparisons between *SMAD6* and non-*SMAD6* controls were 87.4% powered for numerical operations, 92.4% for PIQ, 84.4% for FSIQ, and 74.9% for motor coordination.

fMRI Analysis

Demographics

Thirteen total adolescent craniosynostoses subjects were recruited (Table 10). Six metopic craniosynostosis patients (average age 11.5 years; 4 males, 2 females) and six respective matched controls were included (average age 11.8 years; 4 males, 2 females). Seven ULC patients (average age 12.2; 4 males, 3 females) and seven matched controls were included (average age 12.6; 4 male, 3 female). The average age of surgical correction was 182 days (6.08 months) in the metopic group and 209 days (6.87 months) in the ULC group. Pre-surgical CTs were obtained and analyzed. All metopic patients had moderate frontal stenosis, defined as an endocranial bifrontal angle of 124 to 148° (average 135.8°).[83]

	Metopic	Control	P-value	ULC	Control	P-value
n	6	6		7	7	
Average age (yrs)	11.48 ± 2.4	11.79 ± 2.1	NS	12.20 ± 2.2	12.59 ± 2.5	NS
Gender						
Male	4	4		4	4	
Female	2	2		3	3	
Average age at surgery (days)	182 ± 83.2			209 ± 49.9		
Endocranial Bifrontal Angle	135.76°					
BRIEF Scores						
Emotional	49	54.3	0.3163	42.3	51.7	**0.0652**
Organization of Materials	63	53.8	**0.0534**	46.7	53.5	0.1029

*Approaching significance

Table 10. Subject demographics and functional scores.

Behavioral/Functional Scores

In WISC comparison, the ULC cohort scored significantly higher on verbal comprehension than the metopic cohort (111 ± 2.8 vs 101 ± 3.2, p=0.041), but there were no differences in perceptual reasoning (102 ± 10 vs 114 ± 6.1) , working memory (104 ± 4.3 vs 103 ± 3.3), processing speed (104 ± 4.8 vs 98 ± 5.9), or overall performance (109 ± 1.9 vs 106 ± 4.3).

In BRIEF comparison, metopic patients had higher organization of materials scores, approaching significance, and ULC patients had lower emotional regulation, also approaching but not reaching significance (p=0.053, p=0.065; Table 11).

	Win		Lose		Recovery	
	Time (ms)	P-value	Time (ms)	P-value	Time (ms)	P-value
ULC	723	**<0.0001**	419	**<0.0001**	679	**<0.0001**
ULC Controls	658		398		656	
R-ULC	718	**<0.0001**	428	**<0.0001**	688	**<0.0001**
R-ULC Controls	652		397		647	
Metopic	673	0.8391	407	**0.0407**	660	0.0673
Metopic Controls	672		401		656	

Table 11. Group performance on Win, Lose, Recovery tasks. Performance measured by average time (ms) allowed per image. Stimulus duration is adjusted to allow an error rate of 50 ± 10%. More correct trials led to shortened stimulus time and more incorrect trials led to increased stimulus time. Significance was set at $p<0.05$.

GoNoGo Performance

ULC and R-ULC patients had significantly longer average times, therefore worse performance, than controls ($p<0.0001$) across all conditions. Metopic patients had 6ms longer times during the lose condition than controls ($p=0.041$) but not during other tasks.

fMRI Whole-Brain T-Test and Region of Interest Analysis

Within the metopic group and control group, whole-brain T-test analysis between conditions and non-conditions/resting state were performed at $p<0.05$ and cluster threshold of 675 voxels. This was repeated within the ULC and control groups. Control subject within group T-test results were then subtracted from metopic subject within group T-tests ($p<0.05$ and cluster voxels of 675; Figure 6,7).

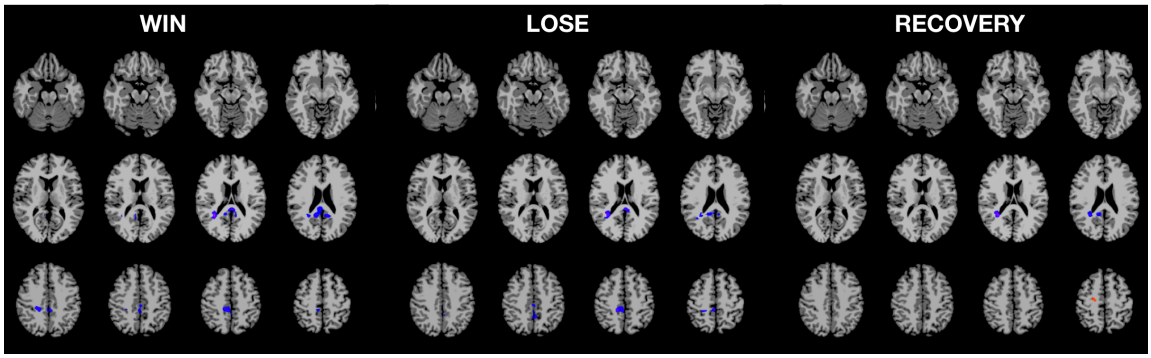

Figure 6. Whole-brain T-Test results for metopic minus controls across conditions "Win", "Lose", and "Recovery".

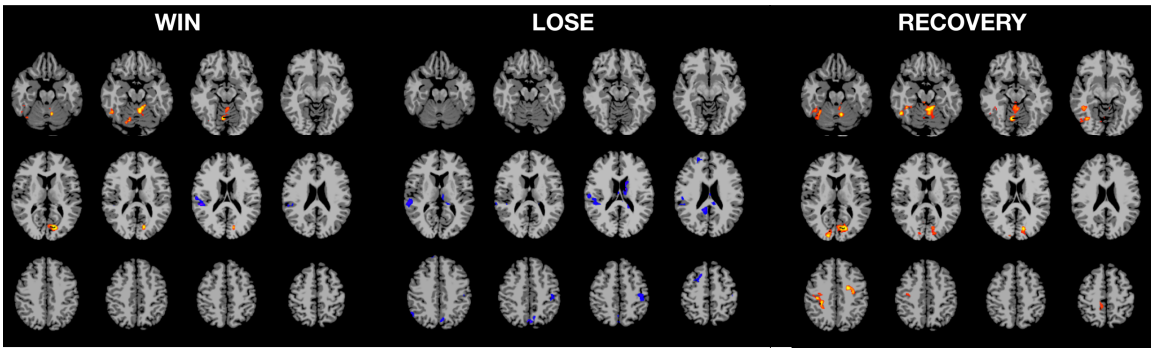

Figure 7. Whole-brain T-Test results for ULC minus controls across conditions "Win", "Lose", and "Recovery".

Using the MNI (Montreal Neurological Institute) stereotaxic space, seeds were isolated and identified in the within group t-test. In the metopic group, four areas of interest were found in the "win" condition, four in the "lose" condition, and two in the "recovery" condition, with few changes in seeds between conditions (Table 12). Among these, all 0/4 seeds had positive T-scores in the "win" condition, 1/4 were positive in the "lose" condition, and 1/2 were positive in the "recovery" condition.

ROI*	Voxels	x	y	z	Max T	BA+	P-value
Metopic WIN							
B/L Superior Posterior Cingulate Gyrus	1664	2.69	-36.45	45.95	-4.320	23, 31	<0.03
B/L Inferior Posterior Cingulate Gyrus	3684	9.06	-46.34	21.65	-7.064	23	<0.03
R Precuneus	931	17.19	-41.75	38.33	-4.377	--	<0.03
R Angular Gyrus and SMG	1173	50.67	-49.42	31.22	-5.345	39	<0.04
Metopic LOSE							
R SFG, MFG, and SMA	1647	16.03	-18.64	65.44	7.139	6	<0.02
L Precuneus	1697	5.03	-38.61	48.51	-6.305	31	<0.03
B/L Posterior Cingulate Gyrus and Splenium	3086	13.12	-46.95	25.71	-5.763	--	<0.04
R Angular Gyrus and SMG	1038	51.38	-47.95	30.56	-5.029	39, 40	<0.04
Metopic RECOVERY							
R SFG, MFG, and SMA	2086	16.06	-18.01	65.08	6.410	6	<0.05
Splenium	1542	17.37	-47.17	22.59	-5.848	23	<0.05

*All anatomical locations found within the ROI
+All Broadman Areas found within the ROI

Table 12. Significant ROIs between metopic patients and controls. Whole brain T-tests between metopic patients minus controls performed between each condition (win, lose, recovery). X,Y,Z coordinates refer to the MNI (Montreal Neurological Institute) stereotaxic space. While-brain analysis threshold set at $p<0.05$; clusters set at ≥ 25. SMG = supramarginal gyrus, SFG = superior frontal gyrus, MFG = middle frontal gyrus, SMA = supplementary motor area.

In the ULC group, six ROIs were found in the "win" condition, eleven in the "lose" condition, and nine in the "recovery" condition, with large fluctuations in seeds between conditions (Table 13). Among these, 1/6 seeds had positive T-scores in the "win" condition, 1/11 were positive in the "lose" condition, and 9/9 were positive in the "recovery" condition.

ROI*	Voxels	x	y	z	Max T	BA+
Unilateral Coronal WIN						
R Cerebelum 7b, 8	951	38.6	-60.45	-46.64	5.119	
R Cerebelum Crus 1, 2 and Cerebelum 6	1157	29.71	-70.58	-32.41	4.283	
L Cerebelum 4, 5, R Cerebelum 6 and Vermis 4, 5, 6	1669	0.71	-58.03	-16.24	5.392	
R ITG and Fusiform Gyrus	1326	42.11	-57.69	-23.82	3.887	37
L Calcarine Sulcus and Cuneus	1144	-11.45	-78.15	10.96	4.369	17, 18, 23
R STG, Insula, and SMG	1040	54.28	-32.19	20.7	-4.886	13, 40, 41
Unilateral Coronal LOSE						
R Cerebelum 7b, 8 and Crus 2	950	38.53	-61.58	-47.71	5.418	
R MTG and STG	1207	59.06	-34.56	8.75	-4.456	22, 41
L MD Nucleus and Pulvinar of the Thalamus	1035	-13.39	-33.15	16.05	-4.186	
R STG, SMG, Postcentral Gyrus, and Insula	1224	54.77	-31.15	22.11	-4.812	41
L VL and VA Nucleus of the Thalamus, Caudate Body, and Corpus Callosum	1109	-8.89	-9.28	19.07	-4.627	
R SFG, MedFG, Anterior Cingulate Gyrus	2066	17.95	48.6	31.13	-5.541	9, 10
R Posterior Cingulate Gyrus and Precuneus	896	12.23	-47.83	25.16	-6.258	23
R Angular Gyrus, SMG, and Inferior Parietal Lobule	1092	55.87	-56.86	37.2	-4.502	39, 40
L Inferior Parietal Lobule and Postcentral Gyrus	940	-43.15	-26.3	48.63	-4.409	2, 3, 4, 40
B/L Precuneus	813	-2.08	-71.22	46.05	-4.623	7
R SFG and SMA	927	16.48	10.23	57.7	-5.286	6
Unilateral Coronal RECOVERY						
R Cerebelum 7b, 8 and Crus 1, 2	1554	39.09	-62.23	-46.88	5.040	
R Cerebelum 6 and Crus 1, 2, Dentate Nuclues, and Vermis 7	3127	24.75	-68.78	-32.2	4.488	
R ITG and Fusiform Gyrus	2226	41.38	-53.77	-19.15	4.969	37
L Cerebelum 3, 4, 5, 6, Lingula, Dentate Nucleus, and Vermis 1, 2, 6	3380	-1.61	-52.61	-17.2	4.728	
R ITG and Fusiform Gyrus	994	35.93	-62.78	-8.72	4.385	19

B/L Lingual Gyrus, Cuneus, and L Calcarine Sulcus	4550	-0.06	-83.87	8.33	5.410	17, 18, 23, 31
L Frontal Lobe Subgyral White Matter	960	-28.48	-5.18	40.23	4.697	6
R Precentral & Postcentral Gyrus and SMG	994	34.12	-27.09	41.81	4.896	2, 3, 4, 6
R Precuneus, Paracentral Lobule, and Postcentral Gyrus	688	8.38	-38.98	58	4.448	4, 5

*All anatomical locations found within the ROI
+All Broadman Areas found within the ROI

Table 13. Significant ROIs between unilateral coronal patients and controls. Whole brain T-tests between unilateral coronal patients minus controls performed between each condition (win, lose, recovery). X,Y,Z coordinates refer to the MNI (Montreal Neurological Institute) stereotaxic space. While-brain analysis threshold set at $p<0.05$; clusters set at ≥ 25. SMG = supramarginal gyrus, SFG = superior frontal gyrus, MFG = middle frontal gyrus, SMA = supplementary motor area, ITG = inferior temporal gyrus, STG = superior temporal gyrus, MTG = middle temporal gyrus, MD = medial dorsal, VL = ventrolateral, VA = ventroanterior, MedFG = medial frontal gyrus.

BOLD Signal Analysis

Brain areas commonly and substantially involved in frustration or anger include the cingulate gyrus, middle temporal gyrus (MTG), Mediodorsal (MD) nucleus of the thalamus, and right cerebellum (Figure 8).[84-86] The cuneus is responsible for primary visual processing but may also have a role in inhibition.[87]

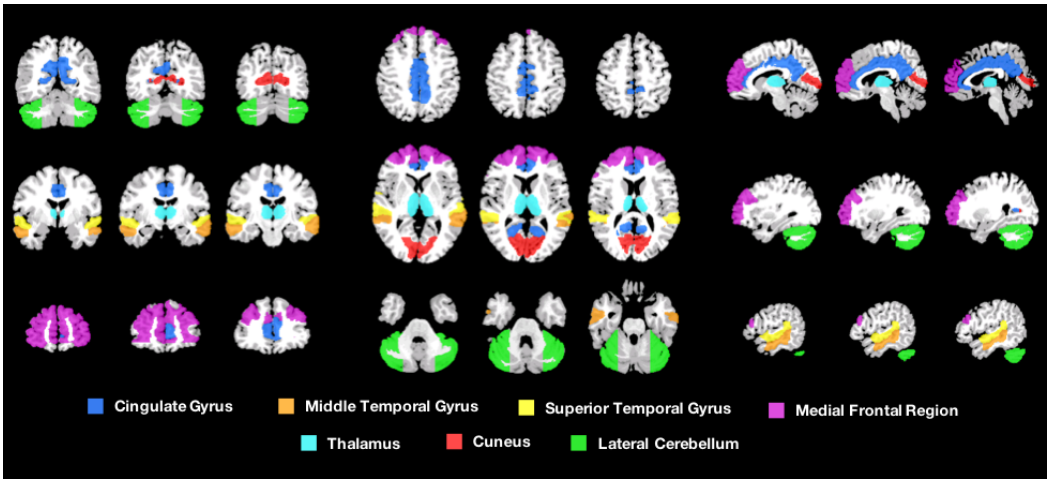

Figure 8. Reference brain for regions in literature substantially involved in frustration.

ROIs identified with seed based analysis were compared between craniosynostosis and controls and between conditions. Metopic patients had decreased signal in right and left posterior cingulate gyrus significantly in the win (p=0.017, p=0.027) and approaching significance in the lose conditions (p=0.062, p=0.075; Figure 9). Right middle temporal gyrus signal was significantly decreased during all three conditions compared to controls (p=0.042, p=0.042, p=0.043), and signal during both the win and recovery tasks were lower than the lose condition (p=0.062, p=0.023).

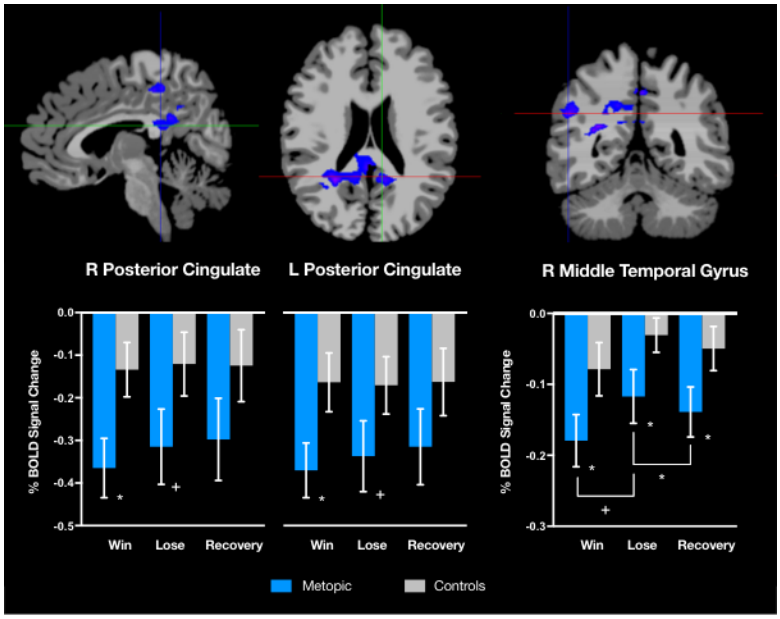

Figure 9. Metopic Synostosis BOLD Signal Comparison. BOLD signal was isolated from ROIs found with seed based analysis. Signal was compared between metopic and control, and between each condition. Error bars indicate standard error. * is significant p<0.05. * between blue and gray bars indicates significant difference between metopic and controls. * with brackets signifies significant group differences (ANOVA) with post-hoc significant difference between indicated conditions (win, lose, recovery).

ULC patients had significantly decreased signal in the right and left posterior cingulate during lose (p=0.023, p=0.031; Figure 10), with significantly lower signal during lose than recovery (p=0.045). Right middle temporal gyrus signal was significantly lower in ULC patients during lose (p=0.032), and on the left was significantly increased during win (p=0.027). Thalamus signal was lower than controls during the lose condition, approaching significance on the right (p=0.088) and significantly on the left (p=0.033), with lower signal during lose than recovery, approaching significance (p=0.080, p=0.064).

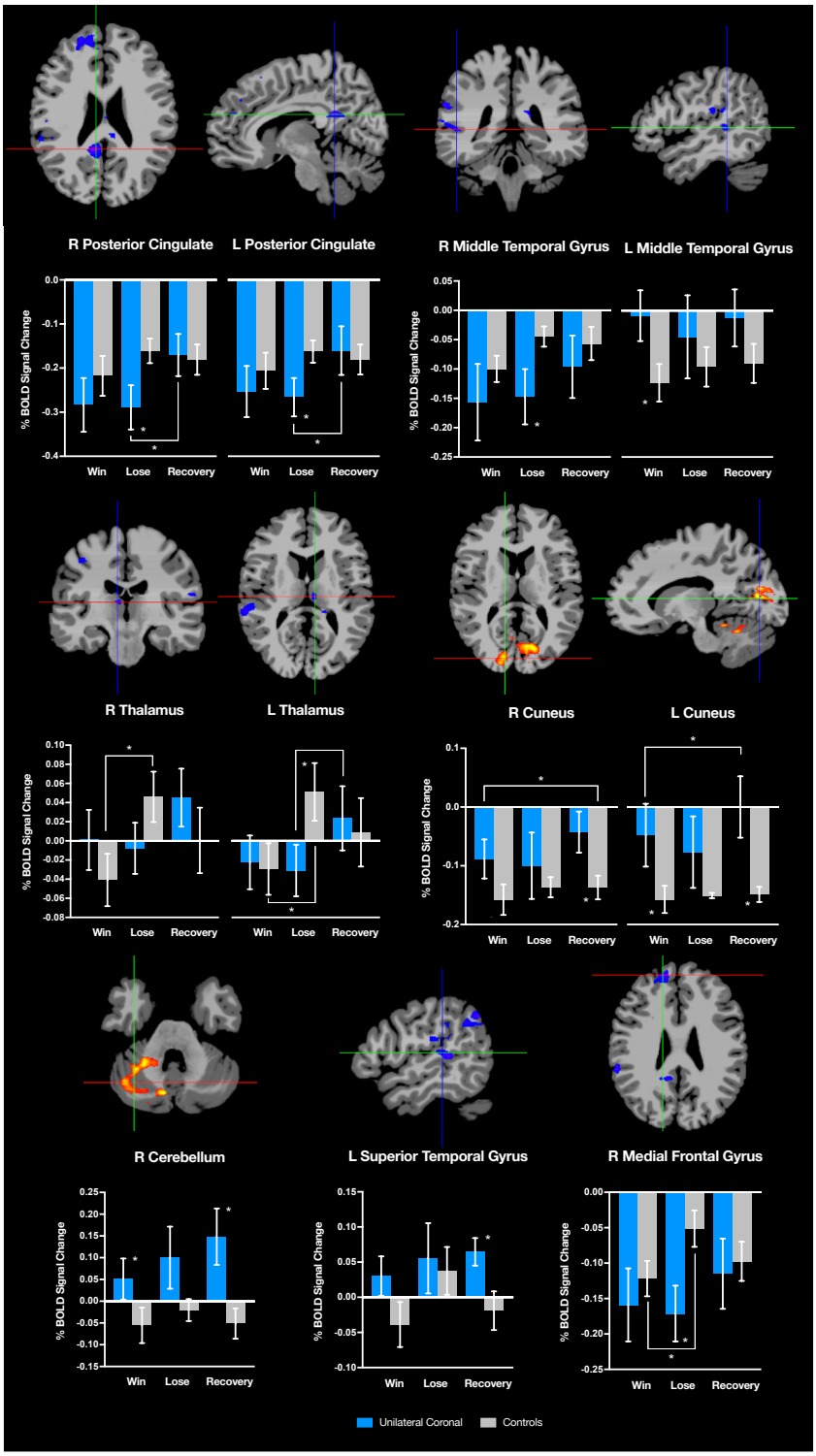

Figure 10. ULC Synostosis BOLD Signal Comparison. BOLD signal was isolated from ROIs found with seed based analysis. Signal was compared between ULC and control,

and between each condition. Error bars indicate standard error. * is significant $p<0.05$. * between blue and gray bars indicates significant difference between ULC and controls. * with brackets signifies significant group differences (ANOVA) with post-hoc significant difference between indicated conditions (win, lose, recovery).

Additionally, in the ULC comparison, right and left cuneus signal was greater than controls during win ($p=0.063$, $p=0.042$) and recovery ($p=0.019$, $p=0.009$), with signal during win significantly decreased compared to that during recovery ($p=0.033$, $p=0.023$). Right cerebellar signal was higher than controls throughout all conditions ($p=0.056$, $p=0.069$, $p=0.009$). Left superior temporal gyrus signal was higher during win ($p=0.065$), approaching significance, and significantly higher during recovery ($p=0.014$). Right MedFG was significantly lower than controls during lose tasks ($p=0.013$).

Subanalysis of R-ULC patients was performed to compare signal in the right and left brain (Figure 11). Right caudate ($p=0.030$), right thalamus ($p=0.011$), and right temporal lobe ($p=0.012$) signal was significantly higher during recovery tasks compared to the right. Right cerebellar signal was significantly higher than the left during all three conditions ($p=0.041$, $p=0.029$, $p=0.033$). No such differences in laterality were observed in controls or metopic patients.

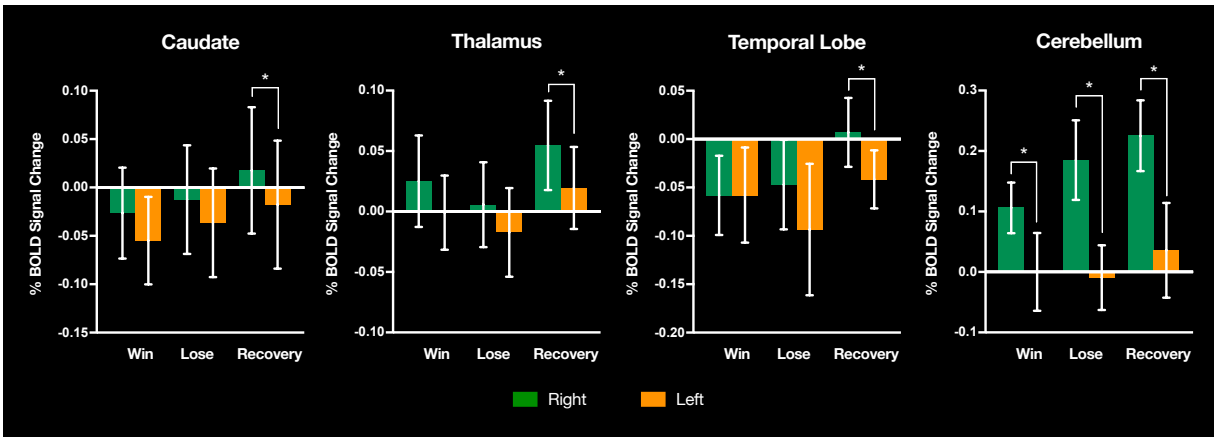

Figure 11. R-ULC Synostosis BOLD signal comparison between left and right. BOLD signal was isolated from ROIs found with seed based analysis. Signal was compared between ROIs in the left and right brain. Error bars indicate standard error. * is significant $p<0.05$. * between blue and gray bars indicates significant difference between R-ULC and controls. * with brackets signifies significant group differences (ANOVA) with post-hoc significant difference between indicated conditions (win, lose, recovery).

ERP and BSID Analysis

Patient Demographics

Twelve nonsyndromic craniosynostosis patients with ERP testing in infancy were neurocognitively tested. One patient was excluded due to a structural brain abnormality found incidentally. The average age of patients during initial infant ERP testing was 7.6 months and the average age at follow up neurocognitive testing was 8.1 years. Thirty three percent of patients were female. With regards to suture fusion, 50% had sagittal synostosis, 25% had metopic, 17% were unilateral coronal, and 8% were combination sagittal and metopic. Ten patients received whole vault cranioplasties and two received endoscopic strip craniectomies.

Neurocognitive Test Performance

On average, patients scored just below the average on the school-age reading/language based neurocognitive assessments (Table 14). Patients scored the in the 44.7th percentile on word reading, 42.0% on reading comprehension, 45.5% in spelling, 44.1% in language composite, and 55.8% verbal IQ.

	Word Reading	Reading Comp	Spelling	Language Composite	Verbal IQ
Standard Score	96.5	90.1	98.8	93.4	102.4
Percentile	44.7	42.0	45.5	44.1	55.8

Table 14. Average language performance of craniosynostosis patients receiving follow up neurocognitive testing.

Neurocognitive Correlation with Infant ERP/BSID Testing

Among all EEG clustered analyzed, only the left frontal cluster MMN output correlated with any neurocognitive outcomes scores (Figure 12). Correlation analyses showed that left frontal cluster MMN strongly correlated with word reading (R 0.713, p=0.031), reading comprehension (R 0.745, p=0.021), and language composite scores (R=0771, p=0.015). No correlation was seen between MMN and spelling or verbal IQ.

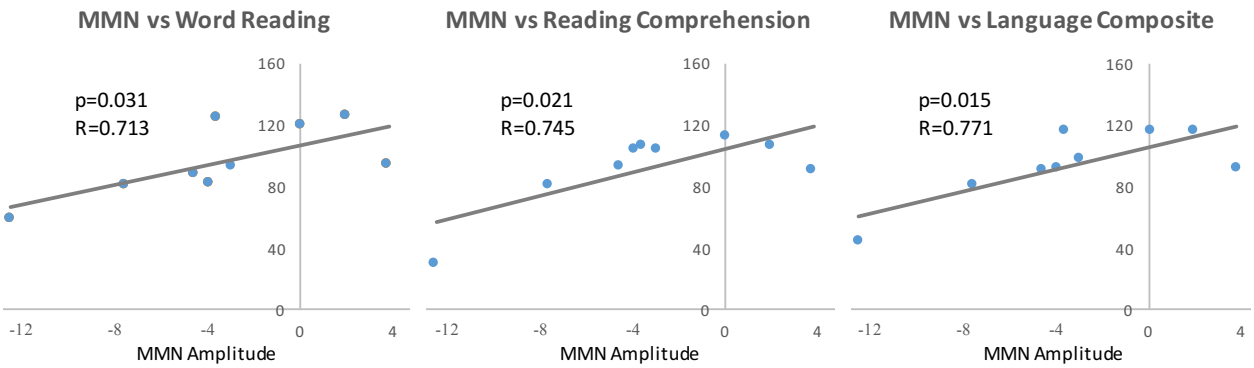

Figure 12. Correlations between infant post-operative left frontal MMN amplitude and school-age language assessment scores.

The highest BSID correlation was expressive language, which actually non-significantly negatively correlating with reading comprehension (R -0.566, p=0.242; Figure 13). BSID cognitive, expressive language, and language composite scores had no predictive value (R<0.5, p>0.05) for school-age neurocognitive scores.

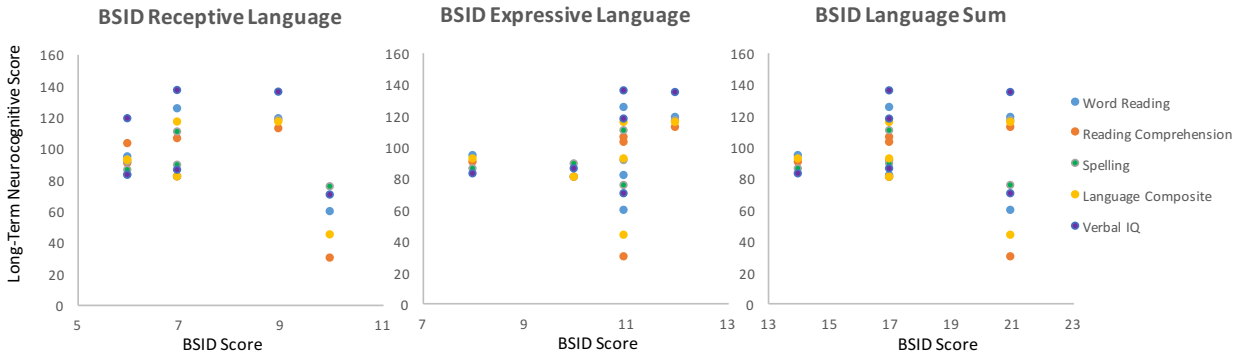

Figure 13. No correlations were found (R<0.50; p>0.05) between infant BSID scores and school-age language assessments.

Controlling for Demographic Confounders

Partial correlations were conducted to exclude demographic factors. Among 22 demographic factors collected, only gestational age correlated with future language composite scores (R 0.778). A partial correlation was conducted between gestational age, left frontal MMN, and language composite scores and, despite this, still supported a moderate to strong correlation between the left frontal MMN and the language composite score (R 0.638).

ERP Comparison between Subtypes of Craniosynostosis

In infancy, 39 controls, 18 sagittal synostosis, 17 metopic, and 6 coronal patients were tested pre and post operatively. The average age at testing for each subtype is represented in Table 15. The amplitude of the left frontal MMN was compared between subtypes pre and post operatively.

	N	Pre-op ERP (Months)	Post-op ERP (Months)
Control	39	6.7	13.2
Sagittal	18	7.1	12.0
Metopic	17	7.6	15.2
Coronal	6	7.7	15.1

Table 15. Average age of participants undergoing pre and post-operative ERP and Bayley's testing.

Pre-operatively, sagittal and metopic patients had significantly attenuated MMN amplitudes when compared to controls (Figure 14). There were no significant differences among subtypes. Post-operatively, sagittal patients no longer had significantly different

MMN amplitudes when compared to controls. Metopic patients still retained significantly attenuated MMN amplitudes compared to controls and became significantly different when compared to sagittal patients as well. Unilateral coronal patients exhibited no difference from the other subtypes.

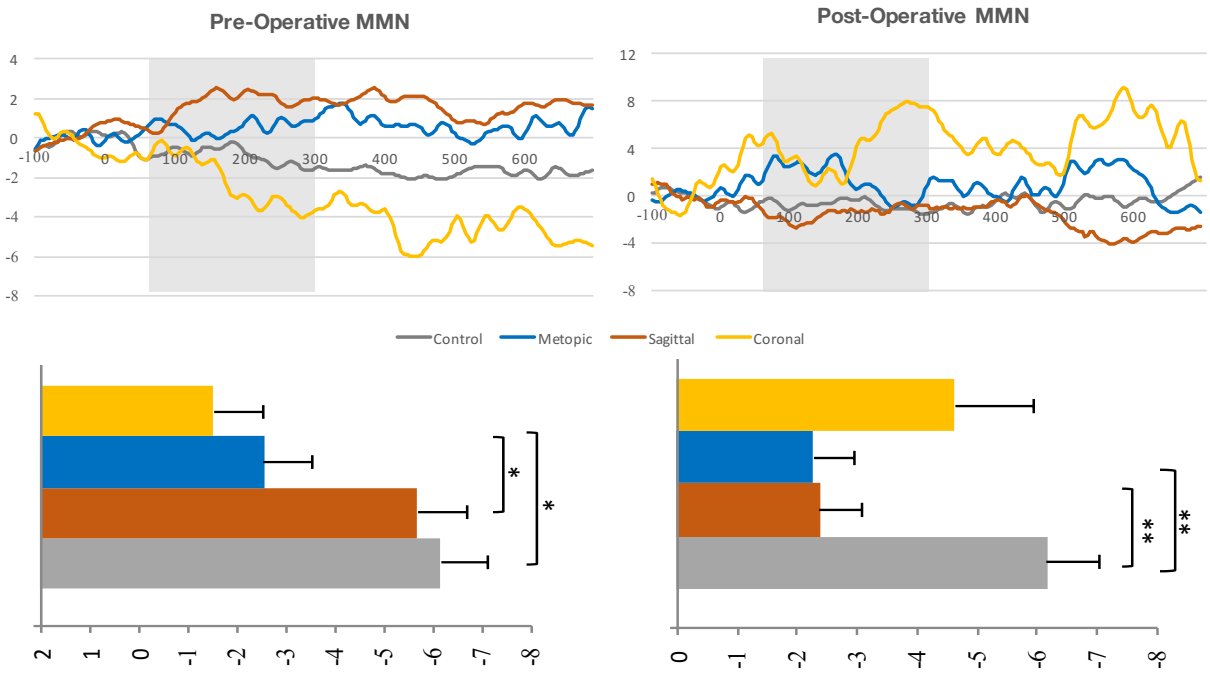

Figure 14. Pre (left) and post (right) operative comparison of MMN amplitudes between subtypes of craniosynostosis and control patients.

Discussion

Unilateral Coronal Craniosynostosis Neurodevelopmental Outcomes

Patients with unilateral coronal synostosis (ULC) comprise up to 20% of nonsyndromic single suture craniosynostosis, yet their long-term neurocognitive profile remains unclear.[88] In this multi-institutional study arm, cranially-mature patients with ULC, subjects had improved language academic achievement in comparison to mathematics, higher VIQ than PIQ, and overall low visuo-motor skills. Breast feeding was particularly influential towards neurodevelopment, along with gender, birth weight, paternal education, and household income. With regards to clinical characteristics, patients with right-sided ULC had a distinct advantage in spelling when compared to left-sided ULC, even when controlling for possible confounding factors.

To the best of our knowledge, our results are the first to identify improved neurocognitive performance with respect to spelling among right ULC patients. Literature suggests varied rates of retardation, IQ, speech-language deficits, and cognition among right versus left coronal craniosynostosis; however, significant differences have never been reported.[38,40]

Kapp-Simon et al. hypothesized that, due to regional brain restriction, left ULC may result in reading-language disorders while right ULC may predispose to nonverbal issues such as social functioning.[49] This may help explain the trend toward improved

language-related performance seen in our data. Lesional studies confirm that left brain damage specifically impacts sublexical spelling ability.[89,90] An fMRI study of school-age ULC subjects indicated asymmetric right-sided hyperemia in the frontal brain, in comparison to controls, endorsing asymmetric neural responses by suture sidedness.[91] This data lends credence to suture stenosis creating regional brain disturbances, thus affecting functional outcomes. Whether this is a product of local brain compression, dural influences, or primary brain malformation is yet to be determined.

Multiple studies of mixed synostoses cohorts demonstrated higher VIQ than PIQ, similar to findings from this cohort, which exhibited an 11 point higher VIQ than PIQ without differences by laterality.[7,92] Our study focused on coronal synostosis in particular, included a broader range of neurocognitive tests, and controlled for 22 relevant demographic factors. Bellew et al. argued that 25% of the normal population are expected to have the VIQ>PIQ discrepancy and proportions above this may prove clinically significant.[92] In our cohort, this discrepancy was identified in 75% of unicoronal patients. In the context of overall normal IQ scores, Magge et al. reasoned this disparity may suggest visuo-motor deficiencies.[7] Such findings were substantiated by below average visuo-motor scores in all three tests administered in our study.

Poor visual-motor performance may be related to orbital dystopia, often resulting in strabismus and astigmatism.[93,94] These findings are unique to ULC synostosis, can persist after surgical correction, and have been reported in up to 90% of patients.[95,96] Denis et al. found high rates of astigmatism, strabismus, and reduced visual acuity for children

operated after 6-7 months of age and normal binocular vision and refraction in all younger surgical patients.[95] Although only trending towards significance, our study showed improved motor coordination in patients operated earlier than 7 months. The significantly worse performance on motor coordination than visual perception and VMI indicates that neural mechanisms in addition to orbital anatomy may play a role.

Neurocognitive studies have varied in the number of control cultural and socioeconomic variables.[28,41] Congruent with other studies, paternal education and household income predicted better performance.[75,76] Breast-feeding was the predominant determinant of VMI, visual perception, and PIQ scores, and was associated with higher averages in all assessments. Although our results only show association, they add to the growing body of literature suggesting the benefit of breast-feeding on IQ, academic achievement, and overall health.[97-99] These three significantly improved cognitive outcomes in breast-fed patients were all measures for which the ULC cohort typically scores lower, suggesting a protective role for breast-feeding in this cohort.

Metopic Craniosynostosis Neurocognitive Outcomes

Increasing evidence across multiple testing modalities has revealed long-term neurocognitive deficits in patients with non-syndromic craniosynostosis. [45,100-102] The majority of studies have been in infants or children with limited analysis of older children and adolescents, ages with improved detection of underlying deficits.

This study arm reveals that the majority of cranially mature patients with surgically corrected metopic craniosynostosis have IQs above national averages and have accompanying academic achievement that is lower and closer to the national means. Previous research has correlated more severe radiographic metopic cases (endocranial bifrontal angles < 124°) with greater dysfunction in the event-related potentials of infants.[48] Our study tested whether metopic severity impacted academic achievement in an older cohort. Academic achievement and reading composite scores remained worse in the severe cohort relative to the moderate patients. Although other measures of academic achievement and IQ were also worse in the severe cohort, these differences were not statistically significant potentially due to limitations in available sample size. This study's findings offer long-term academic and neurocognitive outcomes supporting previous correlations between morphologic severity and test performance.[48]

Event-related potentials (ERP) allow developmental assessments in infants with results that are predictive of future IQ and performance.[48,103-105 106-108] Auditory and visual ERP have previously demonstrate aberrant brainstem responses in syndromic and non-syndromic craniosynostosis.[109-114] Further, more recent published reports have detected higher order language processing abnormalities as early as six months of age in the same patient population.[115] In metopic craniosynostosis, ERP revealed greater attenuation of responses to language stimulation in the frontal cortex in more severe metopic anatomic morphology.[12,48] Our study supports the correlation between phenotypic severity and future achievement in a cranially mature patient population

using validated neurocognitive testing. All neurocognitive testing was performed in a new group of 20 metopic craniosynostosis patients. As the patients from the original ERP and metopic severity scale study reach cranial maturity, future testing will be performed to directly compare phenotypical severity, infant ERP and cranially mature neurocognitive testing in the same patient.

Cranial volume doubles from birth to six months of age and triples by 2.5 years of life.[116,117] Restriction of the growing brain during this period of rapid expansion can lead to localized areas of increased parenchymal pressure, cerebral hypoperfusion, as well as stretching and skewing of white matter tracts during a critical period of synaptogenesis.[118-122] Small disorganizations in synaptogenesis can significantly affect cognitive ability over time.[109,123-126] Our finding that more severe orbito-frontal dysmorphology adversely affect some forms of academic achievement supports this hypothesis.

Sagittal and Metopic SMAD6 Neurocognitive Outcomes

This study arm may present the first link between genotype and neurodevelopmental phenotype in patients with nonsyndromic craniosynostosis. All known school-age patients nationwide with *SMAD6* mutation-influenced craniosynostosis were included in assessment. Midline nonsyndromic craniosynostosis patients with *SMAD6* mutations performed significantly worse on numerical operations, PIQ, FSIQ, and motor coordination than matched midline craniosynostosis controls. Discrepancies persisted

even after controlling for significant external factors. *SMAD6* influenced craniosynostosis patients had worse behavior profiles consisting of worse inhibition, behavior regulation, hyperactivity, aggression, conduct problems, social skills, and communication.

Among eleven assessments, *SMAD6* cases performed below the 50th percentile on eight. However, differences were subtle, most ranging in the 40th-50th percentile. Cognitive delays, even when severe, are difficult to assess prior to school-age peer comparison. When missed, resulting mathematics performance suffers as a function of deficient working memory.[127,128] Similarly, executive function deficits lead to poor behavioral outcomes seen in the parent/guardian reports. Current screening tools, such as the Bayley's Scale of Infant Development, have shown little positive predictive value or can only be utilized in later ages, missing opportunities for remediation.[53] *SMAD6* mutations can cue providers and parents towards mild intellectual delays and provide a basis for early neurocognitive and psychiatric therapy. Genetic testing can be performed even prior to birth, and thus likely represents the earliest screenining tool available to help identify those at higher risk of neurocognitive deficits.[129]

While controls also performed poorly on motor coordination in relation to national averages, *SMAD6* patients performed in the 8th percentile, with the highest in the 18th percentile, almost a standard deviation below control scores. In line with the literature, motor skills in nonsyndromic craniosynostosis appear most susceptible to impairment, even in the absence of cognitive changes.[50,130,131] Hashim et al. reported patients operated

on prior to six months of age also had worse visuomotor integration following strip craniectomy than whole vault cranioplasty.[25] Da Costa et al. identified motor delays persistent motor delays post-operatively, predicted by pre-operative function.[132]

The American Academy of Pediatrics recommends early identification for motor delays.[133] With the absence of clinical validated forms, clinicians rely on parental reports and 9, 18, and 30 month milestones.[134] Genetic testing can be done at any age and results are unequivocal. If *SMAD6* mutations are found, results may advocate for early gross motor remediation.

The etiology of neurodevelopmental delay in nonsyndromic craniosynostosis is unclear. Historical theories argue either increased loco-regional intracranial pressure or secondary cerebral deformation from the overlying cranium might underlie the deficits observed. A third consideration must be given towards primary underlying deformities in the brain, either accompanying suture fusion or as the root cause. This study attempts to lend credence to this theory. Indeed, past research has identified white matter, ventricular, and connectivity differences in patients with nonsyndromic craniosynostosis.[43,135] However, these may develop secondary to calvarial restriction on brain growth.

SMAD6 is an inhibitor of BMP signaling, however it also functions as a mediator of the TGF-ß superfamily.[136] TGF-ß and *SMAD6* have been implicated in the blood brain barrier, fetal neuroprotective mechanisms, and stem cell neurogenesis.[137-139] The extent to

which aberrant BMP and TGF-ß signaling during critical neurodevelopmental periods contribute to intrinsic brain dysfunction remains to be studied.

Neuropsychiatric development occurs in a multifaceted context. Similar to our findings, early age at surgery and early age at neurocognitive evaluation resulted in better performance in nonsyndromic craniosynostosis cohorts.[30,40] Socioeconomic factors were also found to have direct sequela on mental functions in older children.[36] Warschausky et al. found a correlation between maternal education and Bayley's Scale of Infant Development scores, but other studies did not observe similar associations.[36,140] While studies attempt to match between these socioeconomic factors, this study is the first to statistically control for all factors that may contribute to differing development.

fMRI Analysis

This study also presents a characterization of brain connectivity in adolescent nonsyndromic craniosynostosis patients with the first-reported use of task-based fMRI analysis. While both the metopic and coronal sutures are positioned in the frontal cranium, these patient cohorts presented with differential phenotypes and behavioral characteristics. ULC patients experienced lability in response to frustration while metopic patients were comparably unwavering. This was reflected in their fMRI reactivity, task performance, and parental assessments. Several brain regions were identified in metopic patients, including the posterior cingulate gyrus (PCC) and right middle temporal gyrus (MTG) that have differential activation compared to controls. Likewise, ULC had unique

patterns in the PCC, MTG, thalamus, cuneus, right cerebellum, left superior temporal gyrus (STG), and right medial frontal gyrus (MedFG). Differences may take root in brain areas adjacent to suture fusion as the R-ULC group was the only one to display preferential right brain abnormalities.

The right MTG had less deactivation during "lose" than other conditions in the metopic cohort but did not significantly change across conditions in the ULC. While the MTG is known for its role in auditory processing, Bunge et al. correlated right MTG activation with successful NoGo inhibition and Ding et al. implicated hypoactive right MTG activation during NoGo trials in impulsivity of adolescents with gaming addictions.[141,142] This supports the functional findings that metopic patients, with more right MTG activation, performed better on GoNoGo tasks and ULC patients, with consistent right MTG deactivations, may have more impulsivity.

On top of these shared areas, ULC patients had significant differences in several additional brain regions. ULC right cerebellar activations were uniquely present throughout all conditions. While posterior cerebellar activity correlates with GoNogo tasks, fMRI studies have identified the lateral right cerebellar hemisphere to be associated with anger and cognitive components of emotional processing.[85] While most other brain regions were attenuated during "lose", this perhaps explains the disinhibited activity in the right cerebellum. Bilateral thalamic deactivations were seen during the "lose" condition. The MD nucleus of the thalamus sends axons to the limbic system, activating emotional responses. PTSD subjects, another group experiencing emotional

dysregulation, displayed significantly less thalamic and cingulate activation during negative emotional states.[86] While orbital corrections are good, cuneal differences can be traced to subtle visual field modifications, such as astigmatism, often seen in ULC, or may play a role in decreased inhibition.[87,143]

Overall, ULC in this study likely have increased frustration. Deveney et al. reported deactivation in the amygdala, striatum, parietal cortex, and cingulate cortex in irritable children in response to frustrating tasks.[144] The ULC data likewise reflected deactivation in the caudate, putamen, and cingulate cortex during "lose", suggesting chronic irritability. These children may experience frustrating events as more aversive than healthy controls, contributing to inappropriate response.[145]

Metopic patients more likely had stable, if not decreased, reactivity. Bierzynska et al. found that patients with a low tolerance for arousal had precuneus, MFG, and cingulate activation during acute stress, and predicted poor performance in high stimulant environments.[84] Contrasting this, metopic patients had decreased stress activation of these areas, placing them into a high tolerance group. The Default Mode Network, consisting of the precuneus and PCC, is responsible for introspection and self-referential thought, and changes during frustrating tasks suggested exaggerated or attenuated emotional reactivity.[84,146] Taken together, these findings fit the picture that metopic patients showcase fewer negative responses to stressful stimuli.

Finally, laterality differences were observed in R-ULC but not controls or metopic patients. This manifested with increased activity in the temporal lobe, caudate, thalamus,

and cerebellum of the affected side, most prominent during recovery. Many mechanisms could be at play. The release of acute and chronic brain compression results in reactive hyperemia.[147,148] Indeed, David et al. used positron emission computed tomography to image craniosynostosis patients pre and post operatively, and found both increased blood flow and glucose utilization in areas previously compressed by suture fusion 6-12 weeks post-operatively.[149,150] Interestingly, the R-ULC areas involved all play a role in emotional regulation. Hammond posited that transient hyperemia in response to mental strain or emotional disturbance can, over time, permanently distend vessels causing hyper-reactivity in these regions.[151] It is possible that the interplay of chronic reactive hyperemia to surgical decompression and ensuing frustration irregularities may perpetuate increased blood flow to these regions during stress.

ERP and BSID Analysis

Our group was the first to look at ERPs in patients with craniosynostosis. Hashim et al. reported infants with nonsyndromic craniosynostosis have attenuated P150 waves in response to speech sounds compared with normal infants.[24] Yang et al. found that severe metopic synostosis, defined by an endocranial bifrontal angle less than 124^{o}, presented with attenuated P150 waves compared with controls while moderate metopic synostosis (greater than 124^{o}) had no difference. Recent work, not yet published, by Chuang et al. has reanalyzed results looking at the MMN waves pre and post-operatively. Preliminary results found that MMN waves are attenuated preoperatively in sagittal and severe metopic patients but then improve postoperatively.

In this population, we found that left frontal cluster MMN in infancy strongly correlates with future performance in three language-related functional domains. In contrast, BSID, which is widely used to assess developmental progress and predict cognitive development, did not exhibit significant correlation with development in any language-related functional domains. While more work remains to understand the full predictive functions of ERP in cognitive development, ERP shows great potential for use in the clinic. EEG offers an objective, non-invasive, efficient, and relatively inexpensive method for examining developmental changes

ERPs, in particular, can be used to measure changes in brain voltage in response to passive stimuli like the non-native phoneme discrimination paradigm, allowing for detection of abnormalities at a very young age as testing does not require any behavioral input from the participant. Intelligence, attentional, visuospatial components are prevalent in nonsyndromic craniosynostosis patients, however, impairments that relate to learning disabilities may not manifest until children reach school age.[71,73,152] Effective, early detection of neural dysfunction through use of ERPs may help guide treatment earlier, leading to better outcomes.

Furthermore, since EEG equipment is commonly available in many hospital environments, ERP has a relatively easy path to clinical implementation. ERP measurements only take about five minutes to conduct and because the stimulus measures passive response, there is very limited interference from normal ongoing

behavior, which generates a high-quality, objective signal. However, training is required to obtain high quality signals.

Conclusion

We are at the cusp of understanding the neurological impact of nonsyndromic craniosynostosis. Patients with sagittal, unicoronal, metopic (both severe and moderate), and those with particular genetic mutations, represent an eclectic cohort of neuropsychiatric phenotypes. Imaging modalities such as fMRI can help elucidate the exact neuronal connections aberrant to each class of patient. Furthermore, new-age ERP testing can realize direct brain recordings in infants, correlating with future language-based cognitive performance. In the future, we hope to predict individualized neurologic profiles, both pre- and immediately post-natal, with the aim of recommending early targeted neurocognitive intervention, ensuring equal long-term functional outcomes for all patients with nonsyndromic craniosynostosis.

Citations

1. Thorne CH. *Grabb and Smith's plastic surgery.* Lippincott Williams & Wilkins; 2013.
2. Todd TW, Lyon Jr D. Cranial suture closure. Its progress and age relationship. Part II.—Ectocranial closure in adult males of white stock. *American Journal of Physical Anthropology.* 1925;8(1):23-45.
3. Patel A, Terner J, Travieso R, Clune JE, Steinbacher D, Persing JA. On Bernard Sarnat's 100th birthday: pathology and management of craniosynostosis. *Journal of Craniofacial Surgery.* 2012;23(1):105-112.
4. Slater BJ, Lenton KA, Kwan MD, Gupta DM, Wan DC, Longaker MT. Cranial sutures: a brief review. *Plastic and reconstructive surgery.* 2008;121(4):170e-178e.
5. Aldridge K, Kane AA, Marsh JL, Yan P, Govier D, Richtsmeier JT. Relationship of brain and skull in pre-and postoperative sagittal synostosis. *Journal of anatomy.* 2005;206(4):373-385.
6. Aldridge K, Marsh JL, Govier D, Richtsmeier JT. Central nervous system phenotypes in craniosynostosis. *Journal of Anatomy.* 2002;201(1):31-39.
7. Magge SN, Westerveld M, Pruzinsky T, Persing JA. Long-term neuropsychological effects of sagittal craniosynostosis on child development. *Journal of Craniofacial Surgery.* 2002;13(1):99-104.
8. Jane JA, Edgerton MT, Futrell JW, Park TS. Immediate correction of sagittal synostosis. *Journal of neurosurgery.* 1978;49(5):705-710.
9. Posnick JC, Lin KY, Chen P, Armstrong D. Metopic synostosis: quantitative assessment of presenting deformity and surgical results based on CT scans. *Plast Reconstr Surg.* 1994;93(1):16-24.
10. Birgfeld CB, Saltzman BS, Hing AV, et al. Making the diagnosis: metopic ridge versus metopic craniosynostosis. *J Craniofac Surg.* 2013;24(1):178-185.
11. van der Meulen J. Metopic synostosis. *Childs Nerv Syst.* 2012;28(9):1359-1367.
12. Beckett JS, Chadha P, Persing JA, Steinbacher DM. Classification of trigonocephaly in metopic synostosis. *Plast Reconstr Surg.* 2012;130(3):442e-447e.
13. Tan SP, Proctor MR, Mulliken JB, Rogers GF. Early frontofacial symmetry after correction of unilateral coronal synostosis: frontoorbital advancement vs endoscopic strip craniectomy and helmet therapy. *J Craniofac Surg.* 2013;24(4):1190-1194.
14. Oh AK, Wong J, Ohta E, Rogers GF, Deutsch CK, Mulliken JB. Facial asymmetry in unilateral coronal synostosis: long-term results after fronto-orbital advancement. *Plast Reconstr Surg.* 2008;121(2):545-562.
15. Lajeunie E, Le Merrer M, Bonaiti-Pellie C, Marchac D, Renier D. Genetic study of nonsyndromic coronal craniosynostosis. *Am J Med Genet.* 1995;55(4):500-504.
16. Taylor JA, Paliga JT, Wes AM, et al. A critical evaluation of long-term aesthetic outcomes of fronto-orbital advancement and cranial vault remodeling in nonsyndromic unicoronal craniosynostosis. *Plastic and reconstructive surgery.* 2015;135(1):220-231.

17. Di Rocco C, Paternoster G, Caldarelli M, Massimi L, Tamburrini G. Anterior plagiocephaly: epidemiology, clinical findings, diagnosis, and classification. A review. *Child's Nervous System.* 2012;28(9):1413-1422.
18. Huang M, Gruss JS, Clarren SK, et al. The differential diagnosis of posterior plagiocephaly: true lambdoid synostosis versus positional molding. *Plastic and reconstructive surgery.* 1996;98(5):765-774; discussion 775-766.
19. Jimenez DF, Barone CM. Endoscopic craniectomy for early surgical correction of sagittal craniosynostosis. *Journal of neurosurgery.* 1998;88(1):77-81.
20. Utria AF, Mundinger GS, Bellamy JL, et al. The importance of timing in optimizing cranial vault remodeling in syndromic craniosynostosis. *Plastic and reconstructive surgery.* 2015;135(4):1077-1084.
21. Jimenez DF, Barone CM. Multiple-suture nonsyndromic craniosynostosis: early and effective management using endoscopic techniques. *Journal of Neurosurgery: Pediatrics.* 2010;5(3):223-231.
22. Jimenez DF, Barone CM, Cartwright CC, Baker L. Early management of craniosynostosis using endoscopic-assisted strip craniectomies and cranial orthotic molding therapy. *Pediatrics.* 2002;110(1):97-104.
23. Cost H, Project U. Introduction to the HCUP KIDS'inpatient database.
24. Hashim PW, Brooks ED, Persing JA, et al. Direct brain recordings reveal impaired neural function in infants with single-suture craniosynostosis: a future modality for guiding management? *Journal of Craniofacial Surgery.* 2015;26(1):60-63.
25. Hashim PW, Patel A, Yang JF, et al. The effects of whole-vault cranioplasty versus strip craniectomy on long-term neuropsychological outcomes in sagittal craniosynostosis. *Plastic and reconstructive surgery.* 2014;134(3):491-501.
26. Speltz ML, Kapp-Simon KA, Cunningham M, Marsh J, Dawson G. Single-suture craniosynostosis: a review of neurobehavioral research and theory. *Journal of pediatric psychology.* 2004;29(8):651-668.
27. Kapp-Simon KA, Figueroa A, Jocher CA, Schafer M. Longitudinal assessment of mental development in infants with nonsyndromic craniosynostosis with and without cranial release and reconstruction. *Plastic and reconstructive surgery.* 1993;92(5):831-839.
28. Chieffo D, Tamburrini G, Massimi L, et al. Long-term neuropsychological development in single-suture craniosynostosis treated early. *Journal of Neurosurgery: Pediatrics.* 2010;5(3):232-237.
29. Starr JR, Lin HJ, Ruiz-Correa S, et al. Little evidence of association between severity of trigonocephaly and cognitive development in infants with single-suture metopic synostosis. *Neurosurgery.* 2010;67(2):408-416.
30. Patel A, Yang JF, Hashim PW, et al. The impact of age at surgery on long-term neuropsychological outcomes in sagittal craniosynostosis. *Plastic and reconstructive surgery.* 2014;134(4):608e-617e.
31. Shipster C, Hearst D, Somerville A, Stackhouse J, Hayward R, Wade A. Speech, language, and cognitive development in children with isolated sagittal synostosis. *Developmental medicine and child neurology.* 2003;45(1):34-43.

32. Naran S, Miller M, Shakir S, et al. Nonsyndromic craniosynostosis and Associated Abnormal Speech and Language Development. *Plastic and Reconstructive Surgery.* 2017;140(1):62e-69e.
33. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: A latent variable analysis. *Cognitive psychology.* 2000;41(1):49-100.
34. Benton AL. Differential behavioral effects in frontal lobe disease. *Neuropsychologia.* 1968;6(1):53-60.
35. Soares JM, Sampaio A, Marques P, et al. Plasticity of resting state brain networks in recovery from stress. *Frontiers in human neuroscience.* 2013;7.
36. Bottero L, Lajeunie E, Arnaud E, Marchac D, Renier D. Functional outcome after surgery for trigonocephaly. *Plastic and reconstructive Surgery.* 1998;102(4):952-958.
37. Osborn A, Roberts R, Mathias J, Anderson P, Flapper W. Cognitive, behavioral and psychological functioning in children with metopic synostosis: a meta-analysis examining the impact of surgical status. *Child Neuropsychology.* 2018:1-15.
38. Shillito J, Matson DD. Craniosynostosis: a review of 519 surgical patients. *Pediatrics.* 1968;41(4):829-853.
39. Hunter AG, Rudd NL. Craniosynostosis. II. Coronal synostosis: its familial characteristics and associated clinical findings in 109 patients lacking bilateral polysyndactyly or syndactyly. *Teratology.* 1977;15(3):301-309.
40. Becker DB, Petersen JD, Kane AA, Cradock MM, Pilgram TK, Marsh JL. Speech, cognitive, and behavioral outcomes in nonsyndromic craniosynostosis. *Plastic and reconstructive surgery.* 2005;116(2):400-407.
41. Speltz ML, Kapp-Simon K, Collett B, et al. Neurodevelopment of infants with single-suture craniosynostosis: presurgery comparisons with case-matched controls. *Plastic and reconstructive surgery.* 2007;119(6):1874.
42. Aryan HE, Jandial R, Ozgur BM, et al. Surgical correction of metopic synostosis. *Child's Nervous System.* 2005;21(5):392-398.
43. Collmann H, Sörensen N, Krauss J. Hydrocephalus in craniosynostosis: a review. *Child's Nervous System.* 2005;21(10):902-912.
44. Da Costa AC, Anderson VA, Savarirayan R, et al. Neurodevelopmental functioning of infants with untreated single-suture craniosynostosis during early infancy. *Child's Nervous System.* 2012;28(6):869-877.
45. Sidoti EJ, Marsh JL, Marty-Grames L, Noetzel MJ. Long-term studies of metopic synostosis: frequency of cognitive impairment and behavioral disturbances. *Plast Reconstr Surg.* 1996;97(2):276-281.
46. Mendonca DA, White N, West E, Dover S, Solanki G, Nishikawa H. Is there a relationship between the severity of metopic synostosis and speech and language impairments? *Journal of Craniofacial Surgery.* 2009;20(1):85-88.
47. Kunz M, Lehner M, Heger A, et al. Neurodevelopmental and esthetic results in children after surgical correction of metopic suture synostosis: a single institutional experience. *Child's Nervous System.* 2014;30(6):1075-1082.

48. Yang JF, Brooks ED, Hashim PW, et al. The Severity of Deformity in Metopic Craniosynostosis Is Correlated with the Degree of Neurologic Dysfunction. *Plast Reconstr Surg.* 2017;139(2):442-447.
49. Kapp-Simon KA, Speltz ML, Cunningham ML, Patel PK, Tomita T. Neurodevelopment of children with single suture craniosynostosis: a review. *Child's Nervous System.* 2007;23(3):269-281.
50. Kapp-Simon KA, Leroux B, Cunningham M, Speltz ML. Multisite study of infants with single-suture craniosynostosis: preliminary report of presurgery development. *The Cleft palate-craniofacial journal.* 2005;42(4):377-384.
51. Speltz ML, Collett BR, Wallace ER, et al. Intellectual and academic functioning of school-age children with single-suture craniosynostosis. *Pediatrics.* 2015;135(3):e615-e623.
52. Albers CA, Grieve AJ. Test review: Bayley, N.(2006). Bayley scales of infant and toddler development–third edition. San Antonio, TX: Harcourt assessment. *Journal of Psychoeducational Assessment.* 2007;25(2):180-190.
53. Hack M, Taylor HG, Drotar D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. *Pediatrics.* 2005;116(2):333-341.
54. Caviness Jr V, Kennedy D, Richelme C, Rademacher J, Filipek P. The human brain age 7–11 years: a volumetric analysis based on magnetic resonance images. *Cerebral cortex.* 1996;6(5):726-736.
55. Göthlin M, Eckerström M, Rolstad S, Kettunen P, Wallin A. Better prognostic accuracy in younger mild cognitive impairment patients with more years of education. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring.* 2018.
56. Committee on Psychological Testing IV. *Psychological testing in the service of disability determination.* National Academies Press (US); 2015.
57. Timberlake AT, Choi J, Zaidi S, et al. Two locus inheritance of non-syndromic midline craniosynostosis via rare SMAD6 and common BMP2 alleles. *Elife.* 2016;5:e20125.
58. Arnaud E, Meneses P, Lajeunie E, Thorne JA, Marchac D, Renier D. Postoperative mental and morphological outcome for nonsyndromic brachycephaly. *Plastic and reconstructive surgery.* 2002;110(1):6-12; discussion 13.
59. Lelieveld SH, Reijnders MR, Pfundt R, et al. Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nature neuroscience.* 2016;19(9):1194.
60. Beckett JS, Brooks ED, Lacadie C, et al. Altered brain connectivity in sagittal craniosynostosis: Laboratory investigation. *Journal of Neurosurgery: Pediatrics.* 2014;13(6):690-698.
61. Sun AH, Eilbott J, Chuang C, et al. Whole-Brain Intrinsic Connectivity in Nonsyndromic craniosynostosis. *Plastic and Reconstructive Surgery–Global Open.* 2017;5(4S):89.
62. Bayley N. *Bayley scales of infant development: Manual.* Psychological Corporation; 1993.
63. De Haan M. *Infant EEG and event-related potentials.* Psychology Press; 2013.

64. Seery AM, Vogel-Farley V, Tager-Flusberg H, Nelson CA. Atypical lateralization of ERP response to native and non-native speech in infants at risk for autism spectrum disorder. *Developmental cognitive neuroscience.* 2013;5:10-24.
65. Balan P, Kushnerenko E, Sahlin P, Huotilainen M, Näätänen R, Hukki J. Auditory ERPs reveal brain dysfunction in infants with plagiocephaly. *Journal of Craniofacial Surgery.* 2002;13(4):520-525.
66. Näätänen R, Paavilainen P, Reinikainen K. Do event-related potentials to infrequent decrements in duration of auditory stimuli demonstrate a memory trace in man? *Neuroscience letters.* 1989;107(1):347-352.
67. Näätänen R, Paavilainen P, Rinne T, Alho K. The mismatch negativity (MMN) in basic research of central auditory processing: a review. *Clinical Neurophysiology.* 2007;118(12):2544-2590.
68. Jusczyk PW. How infants begin to extract words from speech. *Trends in cognitive sciences.* 1999;3(9):323-328.
69. Swingley D, Aslin RN. Spoken word recognition and lexical representation in very young children. *Cognition.* 2000;76(2):147-166.
70. Näätänen R, Lehtokoski A, Lennes M, et al. Language-specific phoneme representations revealed by electric and magnetic brain responses. *Nature.* 1997;385(6615):432.
71. Kuhl PK. Early language acquisition: cracking the speech code. *Nature Reviews. Neuroscience.* 2004;5(11):831.
72. Jansson-Verkasalo E, Ruusuvirta T, Huotilainen M, et al. Atypical perceptual narrowing in prematurely born infants is associated with compromised language acquisition at 2 years of age. *BMC neuroscience.* 2010;11(1):88.
73. Tsao FM, Liu HM, Kuhl PK. Speech perception in infancy predicts language development in the second year of life: A longitudinal study. *Child development.* 2004;75(4):1067-1084.
74. Hashim PW, Patel A, Yang JF, et al. The effects of whole-vault cranioplasty versus strip craniectomy on long-term neuropsychological outcomes in sagittal craniosynostosis. *Plast Reconstr Surg.* 2014;134(3):491-501.
75. Noble KG, McCandliss BD, Farah MJ. Socioeconomic gradients predict individual differences in neurocognitive abilities. *Developmental science.* 2007;10(4):464-480.
76. Noble KG, Houston SM, Brito NH, et al. Family income, parental education and brain structure in children and adolescents. *Nature neuroscience.* 2015;18(5):773.
77. Noble KG, Norman MF, Farah MJ. Neurocognitive correlates of socioeconomic status in kindergarten children. *Developmental science.* 2005;8(1):74-87.
78. Anderson P, Doyle LW, Group VICS. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *jama.* 2003;289(24):3264-3272.
79. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand K. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *Jama.* 2002;288(6):728-737.
80. Patrick DL, Topolski TD, Edwards TC, et al. Measuring the quality of life of youth with facial differences. *The Cleft palate-craniofacial journal : official*

publication of the American Cleft Palate-Craniofacial Association. 2007;44(5):538-547.
81. Havlik RJ, Azurin DJ, Bartlett SP, Whitaker LA. Analysis and treatment of severe trigonocephaly. *Plast Reconstr Surg.* 1999;103(2):381-390.
82. Simmonds DJ, Pekar JJ, Mostofsky SH. Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent. *Neuropsychologia.* 2008;46(1):224-232.
83. Beckett JS, Chadha P, Persing JA, Steinbacher DM. Classification of trigonocephaly in metopic synostosis. *Plastic and reconstructive surgery.* 2012;130(3):442e-447e.
84. Bierzynska M, Bielecki M, Marchewka A, et al. Effect of frustration on brain activation pattern in subjects with different temperament. *Frontiers in psychology.* 2016;6:1989.
85. Baumann O, Mattingley JB. Functional topography of primary emotion processing in the human cerebellum. *NeuroImage.* 2012;61(4):805-811.
86. Lanius RA, Williamson PC, Hopper J, et al. Recall of emotional states in posttraumatic stress disorder: an fMRI investigation. *Biological psychiatry.* 2003;53(3):204-210.
87. Haldane M, Cunningham G, Androutsos C, Frangou S. Structural brain correlates of response inhibition in Bipolar Disorder I. *Journal of Psychopharmacology.* 2008;22(2):138-143.
88. Di Rocco F, Arnaud E, Renier D. Evolution in the frequency of nonsyndromic craniosynostosis. *Journal of Neurosurgery: Pediatrics.* 2009;4(1):21-25.
89. Rapcsak SZ, Beeson PM. The role of left posterior inferior temporal cortex in spelling. *Neurology.* 2004;62(12):2221-2229.
90. Henry ML, Beeson PM, Stark AJ, Rapcsak SZ. The role of left perisylvian cortical regions in spelling. *Brain and language.* 2007;100(1):44-52.
91. Wu RT, Yang JF, Zucconi WB, et al. Frustration and Emotional Regulation in Nonsyndromic craniosynostosis: an fMRI Study. *Plastic and Reconstructive Surgery Global Open.* 2018;6(4 Suppl).
92. Bellew M, Chumas P. Long-term developmental follow-up in children with nonsyndromic craniosynostosis. *Journal of Neurosurgery: Pediatrics.* 2015;16(4):445-451.
93. Baranello G, Vasco G, Ricci D, Mercuri E. Visual function in nonsyndromic craniosynostosis: past, present, and future. *Child's Nervous System.* 2007;23(12):1461-1465.
94. Beckett JS, Persing JA, Steinbacher DM. Bilateral orbital dysmorphology in unicoronal synostosis. *Plastic and reconstructive surgery.* 2013;131(1):125-130.
95. Denis D, Genitori L, Bolufer A, Lena G, Saracco JB, Choux M. Refractive error and ocular motility in plagiocephaly. *Child's Nervous System.* 1994;10(4):210-216.
96. Diamond GR, Katowitz JA, Whitaker LA, Bersani TA, Bartlett SP, Welsh MG. Ocular and adnexal complications of unilateral orbital advancement for plagiocephaly. *Archives of Ophthalmology.* 1987;105(3):381-385.

97. Horwood L, Darlow B, Mogridge N. Breast milk feeding and cognitive ability at 7–8 years. *Archives of Disease in Childhood-Fetal and Neonatal Edition.* 2001;84(1):F23-F27.
98. Horwood LJ, Fergusson DM. Breastfeeding and later cognitive and academic outcomes. *Pediatrics.* 1998;101(1):e9-e9.
99. Chung M, Raman G, Chew P, Magula N, Trikalinos T, Lau J. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Technol Asses (Full Rep).* 2007;153(153):1-186.
100. Bottero L, Lajeunie E, Arnaud E, Marchac D, Renier D. Functional outcome after surgery for trigonocephaly. *Plast Reconstr Surg.* 1998;102(4):952-958; discussion 959-960.
101. Magge SN, Westerveld M, Pruzinsky T, Persing JA. Long-term neuropsychological effects of sagittal craniosynostosis on child development. *J Craniofac Surg.* 2002;13(1):99-104.
102. Becker DB, Petersen JD, Kane AA, Cradock MM, Pilgram TK, Marsh JL. Speech, cognitive, and behavioral outcomes in nonsyndromic craniosynostosis. *Plast Reconstr Surg.* 2005;116(2):400-407.
103. SJ. L. *An Introduction to the Event-Related Potential Technique.* Cambridge, MA: A Bradford Book; 2014.
104. Makeig S, Westerfield M, Jung TP, et al. Functionally independent components of the late positive event-related potential during visual spatial attention. *J Neurosci.* 1999;19(7):2665-2680.
105. Makeig S, Westerfield M, Townsend J, Jung TP, Courchesne E, Sejnowski TJ. Functionally independent components of early event-related potentials in a visual spatial attention task. *Philos Trans R Soc Lond B Biol Sci.* 1999;354(1387):1135-1144.
106. Molfese DL. Predicting dyslexia at 8 years of age using neonatal brain responses. *Brain Lang.* 2000;72(3):238-245.
107. Guttorm TK, Leppanen PH, Hamalainen JA, Eklund KM, Lyytinen HJ. Newborn event-related potentials predict poorer pre-reading skills in children at risk for dyslexia. *J Learn Disabil.* 2010;43(5):391-401.
108. Leppanen PH, Hamalainen JA, Salminen HK, et al. Newborn brain event-related potentials revealing atypical processing of sound frequency and the subsequent association with later literacy skills in children with familial dyslexia. *Cortex.* 2010;46(10):1362-1376.
109. Brooks ED, Beckett JS, Yang J, et al. The Etiology of Neuronal Development in Craniosynostosis: A Working Hypothesis. *J Craniofac Surg.* 2018;29(1):49-55.
110. Church MW, Parent-Jenkins L, Rozzelle AA, Eldis FE, Kazzi SN. Auditory brainstem response abnormalities and hearing loss in children with craniosynostosis. *Pediatrics.* 2007;119(6):e1351-1360.
111. Donati R, Landi A, Rovati LC, et al. Neurophysiological evaluation with multimodality evoked potentials in craniostenosis and craniofacial stenosis. *J Craniofac Surg.* 1997;8(4):286-289.
112. Liasis A, Nischal KK, Walters B, et al. Monitoring visual function in children with syndromic craniosynostosis: a comparison of 3 methods. *Arch Ophthalmol.* 2006;124(8):1119-1126.

113. Mursch K, Brockmann K, Lang JK, Markakis E, Behnke-Mursch J. Visually evoked potentials in 52 children requiring operative repair of craniosynostosis. *Pediatr Neurosurg.* 1998;29(6):320-323.
114. Thompson DA, Liasis A, Hardy S, et al. Prevalence of abnormal pattern reversal visual evoked potentials in craniosynostosis. *Plast Reconstr Surg.* 2006;118(1):184-192.
115. Hashim PW, Brooks ED, Persing JA, et al. Direct brain recordings reveal impaired neural function in infants with single-suture craniosynostosis: a future modality for guiding management? *J Craniofac Surg.* 2015;26(1):60-63.
116. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychol Rev.* 2010;20(4):327-348.
117. Iwasaki N, Hamano K, Okada Y, et al. Volumetric quantification of brain development using MRI. *Neuroradiology.* 1997;39(12):841-846.
118. Persing JA, Jane JA, Shaffrey M. Virchow and the pathogenesis of craniosynostosis: a translation of his original work. *Plast Reconstr Surg.* 1989;83(4):738-742.
119. Aldridge K, Marsh JL, Govier D, Richtsmeier JT. Central nervous system phenotypes in craniosynostosis. *J Anat.* 2002;201(1):31-39.
120. Huisman TA, Martin E, Kubik-Huch R, Marincek B. Fetal magnetic resonance imaging of the brain: technical considerations and normal brain development. *Eur Radiol.* 2002;12(8):1941-1951.
121. Garel C, Chantrel E, Brisse H, et al. Fetal cerebral cortex: normal gestational landmarks identified using prenatal MR imaging. *AJNR Am J Neuroradiol.* 2001;22(1):184-189.
122. Tau GZ, Peterson BS. Normal development of brain circuits. *Neuropsychopharmacology.* 2010;35(1):147-168.
123. Kapp-Simon KA, Speltz ML, Cunningham ML, Patel PK, Tomita T. Neurodevelopment of children with single suture craniosynostosis: a review. *Childs Nerv Syst.* 2007;23(3):269-281.
124. Churchill JD, Beckel-Mitchener A, Weiler IJ, Greenough WT. Effects of Fragile X syndrome and an FMR1 knockout mouse model on forebrain neuronal cell biology. *Microsc Res Tech.* 2002;57(3):156-158.
125. Morton JB, Munakata Y. What's the difference? Contrasting modular and neural network approaches to understanding developmental variability. *J Dev Behav Pediatr.* 2005;26(2):128-139.
126. Munakata Y, Casey BJ, Diamond A. Developmental cognitive neuroscience: progress and potential. *Trends Cogn Sci.* 2004;8(3):122-128.
127. Bull R, Scerif G. Executive functioning as a predictor of children's mathematics ability: Inhibition, switching, and working memory. *Developmental neuropsychology.* 2001;19(3):273-293.
128. Berg DH. Working memory and arithmetic calculation in children: The contributory roles of processing speed, short-term memory, and reading. *Journal of experimental child psychology.* 2008;99(4):288-308.
129. Timberlake AT, Persing JA. Genetics of Nonsyndromic craniosynostosis. *Plastic and reconstructive surgery.* 2018;141(6):1508-1516.

130. Cohen SR, Cho DC, Nichols SL, Simms C, Cross KP, Burstein FD. American Society of Maxillofacial Surgeons outcome study: preoperative and postoperative neurodevelopmental findings in single-suture craniosynostosis. *Plastic and reconstructive surgery.* 2004;114(4):841-847.
131. Bellew M, Chumas P, Mueller R, Liddington M, Russell J. Pre-and postoperative developmental attainment in sagittal synostosis. *Archives of disease in childhood.* 2005;90(4):346-350.
132. Da Costa AC, Anderson VA, Holmes AD, et al. Longitudinal study of the neurodevelopmental characteristics of treated and untreated nonsyndromic craniosynostosis in infancy. *Child's Nervous System.* 2013;29(6):985-995.
133. Noritz GH, Murphy NA. Motor delays: early identification and evaluation. *Pediatrics.* 2013:peds. 2013-1056.
134. Feldman DE, Couture M, Grilli L, Simard M-N, Azoulay L, Gosselin J. When and by whom is concern first expressed for children with neuromotor problems? *Archives of pediatrics & adolescent medicine.* 2005;159(9):882-886.
135. Raybaud C, Di Rocco C. Brain malformation in syndromic craniosynostoses, a primary disorder of white matter: a review. *Child's Nervous System.* 2007;23(12):1379-1388.
136. Imamura T, Takase M, Nishihara A, et al. Smad6 inhibits signalling by the TGF-β superfamily. *Nature.* 1997;389(6651):622.
137. Rodriguez-Martinez G, Velasco I. Activin and TGF-β effects on brain development and neural stem cells. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders).* 2012;11(7):844-855.
138. Cox-Limpens KE, Vles JS, Schlechter J, Zimmermann LJ, Strackx E, Gavilanes AW. Fetal brain genomic reprogramming following asphyctic preconditioning. *BMC neuroscience.* 2013;14(1):61.
139. Engelhardt B. Development of the blood-brain barrier. *Cell and tissue research.* 2003;314(1):119-129.
140. Warschausky S, Angobaldo J, Kewman D, Buchman S, Muraszko KM, Azengart A. Early development of infants with untreated metopic craniosynostosis. *Plastic and reconstructive surgery.* 2005;115(6):1518-1523.
141. Ding W-n, Sun J-h, Sun Y-w, et al. Trait impulsivity and impaired prefrontal impulse inhibition function in adolescents with internet gaming addiction revealed by a Go/No-Go fMRI study. *Behavioral and Brain Functions.* 2014;10(1):20.
142. Bunge SA, Dudukovic NM, Thomason ME, Vaidya CJ, Gabrieli JD. Immature frontal lobe contributions to cognitive control in children: evidence from fMRI. *Neuron.* 2002;33(2):301-311.
143. Levy RL, Rogers GF, Mulliken JB, Proctor MR, Dagi LR. Astigmatism in unilateral coronal synostosis: incidence and laterality. *Journal of American Association for Pediatric Ophthalmology and Strabismus {JAAPOS}.* 2007;11(4):367-372.
144. Deveney CM, Connolly ME, Haring CT, et al. Neural mechanisms of frustration in chronically irritable children. *American Journal of Psychiatry.* 2013;170(10):1186-1194.

145. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science.* 1997;275(5306):1593-1599.
146. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proceedings of the National Academy of Sciences.* 2001;98(2):676-682.
147. Griffiths IR. Vasogenic edema following acute and chronic spinal cord compression in the dog. *Journal of neurosurgery.* 1975;42(2):155-165.
148. Miller JD, Stanek AE, Langfitt TW. Cerebral blood flow regulation during experimental brain compression. *Journal of neurosurgery.* 1973;39(2):186-196.
149. David LR, Genecov DG, Camastra AA, Wilson JA, Argenta LC. Positron emission tomography studies confirm the need for early surgical intervention in patients with single-suture craniosynostosis. *The Journal of craniofacial surgery.* 1999;10(1):38-42.
150. David LR, Wilson JA, Watson NE, Argenta LC. Cerebral perfusion defects secondary to simple craniosynostosis. *The Journal of craniofacial surgery.* 1996;7(3):177-185.
151. Hammond WA. *Cerebral hyperaemia, the result of mental strain or emotional disturbance.* Brentano's; 1895.
152. Molfese DL. Predicting dyslexia at 8 years of age using neonatal brain responses. *Brain and language.* 2000;72(3):238-245.